



Published in final edited form as:

Nat Rev Cardiol. 2018 September ; 15(9): 555–565. doi:10.1038/s41569-018-0030-z.

Endothelial dysfunction and angiogenesis impairment in the ageing vasculature

Zoltan Ungvari^{1,2,3}, Stefano Tarantini^{1,2,3}, Tamas Kiss^{1,2,3}, Jonathan D. Wren^{1,4}, Cory B. Giles^{1,4}, Courtney T. Griffin⁵, Walter Lee Murfee⁶, Pal Pacher⁷, and Anna Csizsar^{1,2,3}

¹Reynolds Oklahoma Center on Aging, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA

²Translational Geroscience Laboratory, Department of Geriatric Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA

³Department of Medical Physics and Informatics, Faculty of Medicine and Faculty of Science and Informatics, University of Szeged, Szeged, Hungary 6720

⁴Arthritis & Clinical Immunology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, USA

⁵Cardiovascular Biology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, USA

⁶J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, Gainesville, FL 32611, USA

⁷Laboratory of Cardiovascular Physiology and Tissue Injury, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, USA

Abstract

Aging is the main risk factor for the development of cardiovascular diseases. A key mechanism by which aging promotes vascular pathologies is by compromising endothelial health. Age-related attenuation of endothelium-dependent dilator responses (‘endothelial dysfunction’) associated with impairment of angiogenic processes and subsequent pathologic remodeling of the microcirculation contribute to compromised tissue perfusion and exacerbates functional decline in older subjects. This brief overview focuses on cellular, molecular, and functional changes that occur in the endothelium during aging. It explores the links between oxidative/nitrative stress and conserved molecular pathways impacting endothelial dysfunction and impaired angiogenesis during aging. This review also speculates on how these pathological processes could be therapeutically targeted. Improved understanding of endothelial biology in older patients is crucial for all cardiologists, as maintenance of a competently functioning endothelium is critical for adequate tissue perfusion and long-term cardiac health.

INTRODUCTION

In Europe and Northern America more than one in five persons is aged 65 or over, and this cohort will reach 30% by mid-century in many developed countries¹. Cardiovascular diseases (CVD) is the foremost cause of death and disability among the elderly in the Western world¹. CVD is a disease of aging; currently approximately 66% of CVDs occur in subjects 75 years of age or more¹. Age-specific mortality rates from CVD exponentially increase with age throughout the later years of life¹. It is becoming evident that aging results in well-defined phenotypic changes, which render the coronary circulation prone to disease even in the absence of traditional risk factors (e.g. hypertension, metabolic diseases, and smoking). For example, aging results in complex dysregulation of microvascular perfusion, induces pro-atherogenic and pro-inflammatory effects, alterations in the extracellular matrix that impact vascular stability, and changes in the secretory, transport, and barrier functions of vascular cells. Understanding the mechanisms implicated in age-related impairment of the coronary circulation are essential for reducing cardiovascular mortality in an aging population. In this review, the effect of aging on the functional and structural integrity of the coronary circulation is considered in terms of potential mechanisms involved in endothelial dysfunction and age-related impairment of angiogenesis. The consequences of combined functional and structural impairment of the coronary circulation are discussed.

ENDOTHELIAL DYSFUNCTION IN AGING

A large body of evidence suggests that impaired endothelium-dependent nitric oxide (NO)-mediated vasodilation (“endothelial dysfunction”) is associated with cardiovascular events (reviewed in²). Clinical and preclinical studies demonstrate that aging is a major cause for endothelial dysfunction³⁻⁵. Endothelial dysfunction is an early feature of atherosclerotic vascular diseases and significantly contributes to impaired microvascular perfusion. It is well established that endothelium-derived NO is a critical vasodilator that regulates vascular resistance and tissue perfusion. In addition to maintaining normal organ blood flow, endothelium-derived NO also exerts significant vasoprotective and cardioprotective effects. For example, NO regulates cell division and survival, inhibits platelet aggregation and inflammatory cell adhesion to endothelial cells, disrupts pro-inflammatory cytokine-induced signaling pathways, preserves endothelial progenitor cell function, and regulates mitochondrial function and cellular energy metabolism¹. Endothelial dysfunction and consequential impaired bioavailability of NO promote the pathogenesis of CVD and hypertension, vascular cognitive impairment, and a range of pathological conditions from erectile dysfunction to claudication. The critical role of endothelium-derived NO in cardiovascular protection in aging is underscored by the findings that endothelial nitric oxide synthase (eNOS)-deficient mice exhibit a premature cardiac aging phenotype associated with early mortality⁶. The mechanisms underlying age-related endothelial dysfunction are multifaceted and likely involve increased oxidative/nitrative stress and alterations in conserved molecular pathways impacting common aging processes (Figure 1).

Role of oxidative stress

The free radical theory of aging, first proposed by Harman⁷, postulates that age-related physiological decline is determined by the endogenous production of reactive oxygen species (ROS) in cells, resulting in cumulative macromolecular damage. Although the role of ROS in regulation of lifespan is still hotly debated, key components of the theory have been substantiated. Since the early 2000s, several groups have reported that increased levels of ROS contribute significantly to vascular aging by promoting endothelial dysfunction in both aged laboratory animals⁴ and older adults^{8,9}. One of the functional consequences of increased ROS production in aged endothelial cells is inactivation of NO and subsequent vasomotor dysfunction^{4,10-12}. Impaired bioavailability of NO due to age related oxidative stress severely impairs flow/shear stress-induced vasodilation in the coronary circulation⁴, which is a critical mechanism for maintaining normal myocardial perfusion. The severe generalized impairment of NO bioavailability in aging¹³ is also likely exacerbated by an age-related dysregulation of eNOS expression^{4,14-17}, decreased availability of tetrahydrobiopterin¹⁸ and/or a reduced intracellular L-arginine availability¹⁹.

Age-related oxidative stress in the coronary vasculature is, at least in part, due to an increased activity of NAD(P)H oxidase (NOX)^{4,10}. Accordingly, treatments that attenuate NOX activity improve endothelial function in aged coronary arteries⁴. Previous studies suggest that up-regulation of endothelial NOX in the aged vasculature may be due to the increased presence of pro-inflammatory mediators²⁰ and/or increased activity of the renin-angiotensin system²¹. In addition to promoting endothelial dysfunction, NOX may also play a central role in the pathogenesis of atherosclerosis and hypertension through activation of redox signaling pathways.

There is also growing evidence that increased mitochondrial oxidative stress significantly contributes to age-related endothelial dysfunction. With advanced age, increased mitochondria-derived production of ROS can be detected in all tissues, including those in the cardiovascular system²². Recent studies show that treatment with mitochondrial-targeted antioxidants improves endothelial function in aged mice^{23,24}. Likewise, attenuation of mitochondrial oxidative stress in mice by overexpression of human catalase in the mitochondria (MCAT) also delays age-related cardiac pathology²⁵ and improves endothelial function (Csiszar and Ungvari, unpublished observation 2017). Conversely, genetic deletion of the mitochondrial antioxidant proteins superoxide dismutase 2 (SOD2)²⁶ and glutathione peroxidase 1 (GPX1)²⁷ exacerbates age-related vascular dysfunction. The mechanisms promoting mitochondrial oxidative stress in aged endothelial cells are likely multifaceted and may include dysregulation of the electron transport chain²⁸, peroxynitrite-mediated nitration and inactivation of manganese superoxide dismutase (MnSOD)²⁹, decline in glutathione content³⁰, or increased activity of the renin-angiotensin system. Additionally, the NAD(P)H oxidase isoform NOX4 is localized to the mitochondria, and an age-related increase in expression/activity of NOX4 may contribute significantly to mitochondrial oxidative stress²². Likewise, a decline in endothelial mitochondria content due to impaired mitochondrial biogenesis^{28,31,32} may also contribute to increased mitochondria-derived ROS production.

The redox-sensitive transcription factor NF-E2-related factor 2 (Nrf2) plays a critical role in preserving a youthful endothelial phenotype. In young vessels, increased levels of ROS (e.g. induced by metabolic diseases, hypertension) activates adaptive mechanisms that involve induction of Nrf2-driven antioxidant defense mechanisms³³. Nrf2 orchestrates the transcriptional response of endothelial cells to oxidative stress, up-regulating numerous proteins that detoxify ROS, as well as those with other antioxidant properties. There is strong evidence that age-related vascular oxidative stress is exacerbated by a homeostatic failure due to dysregulation of the Nrf2-mediated antioxidant response both in rodent models and non-human primates^{34,35}.

Strong evidence suggests a potential anti-aging role for the gaseotransmitter hydrogen sulfide (H₂S) produced by the evolutionary conserved transsulfuration pathway³⁶. Endogenous H₂S exerts vasoprotective effects, promoting vasodilation, regulating endothelial barrier function³⁷, inducing SIRT1 activation, up-regulating Nrf2 and reducing mitochondrial oxidative stress³⁶. Production of H₂S decreases in aging and is increased by anti-aging interventions including caloric restriction and methionine restriction³⁸.

There is an emerging view, which combines aspects of both the free radical theory of aging and the ‘inflammaging’ theory of chronic inflammation promoting aging related pathologies. ROS, in addition to inactivating NO and causing oxidative macromolecular damage may also have critical signaling roles in the vasculature. For example, both NOX- and mitochondria-derived ROS may be implicated in the pathogenesis of chronic, low grade, sterile vascular inflammation, by activating NF- κ B and other pro-inflammatory signaling mechanisms, up-regulating vascular production of cytokines and chemokines, and promoting endothelial activation and acquisition of a senescent endothelial phenotype^{22,39–41}.

Age-related endothelial dysfunction can be reversed by habitual physical activity, which likely contributes to a reduced risk of cardiovascular disease associated with exercise⁴². The molecular mechanisms of endothelial protection conferred by exercise are likely complex and include a significant reduction of oxidative stress, mitochondrial protection and anti-inflammatory effects^{1,18,43–47}.

Role of nitrosative and nitrative stress

Many of the adverse consequences of oxidative stress are mediated via diffusion limited reaction of free radicals with NO. One of the most studied and accepted physiological gateway to produce reactive nitrogen species (RNS) is through the diffusion-limited reaction of NO with superoxide, which leads to formation of the highly reactive oxidant peroxynitrite⁴⁸. Numerous preclinical studies demonstrated enhanced cardiovascular peroxynitrite generation or consequent protein nitration, and implicated these processes in aging-associated cardiovascular dysfunction^{4,10–12,49,50}. Peroxynitrite may facilitate cardiovascular dysfunction in aging via multiple mechanisms including enhanced lipid peroxidation, enhanced protein oxidation and nitration leading to inactivation of key proteins/enzymes involved in regulation of vascular function (e.g. proteins involved in mitochondrial electron transfer, Ca²⁺ handling, NO signaling), increased LDL oxidation promoting atherogenesis, and activation of stress signaling pathways and matrix metalloproteinases facilitating cell death and pathological remodeling⁴⁸. Peroxynitrite, in

concert with other oxidants, induces strand breaks in DNA, activating the nuclear DNA repair enzyme poly(ADP-ribose) polymerase-1 (PARP-1), which transfers ADP-ribose units from nicotinamide adenine dinucleotide (NAD⁺) to nuclear proteins, including transcription factors and histones, resulting in depletion of the intracellular NAD⁺ pools, slowing the rate of glycolysis and mitochondrial respiration, eventually culminating into cellular dysfunction^{51–53}. Since both SIRT1 and PARP-1 are NAD⁺ utilizing enzymes, induction of PARP-1 decreases SIRT1 activity by consuming cellular NAD⁺ exacerbating vascular aging processes in experimental animals⁴¹. PARP-1 activation, in part via inhibiting SIRT1, has been shown to modulate the transcriptional regulation of various pro-inflammatory genes^{54,55} such as NF- κ B activation^{56–58}. The peroxynitrite-PARP-1 pathway has also been implicated in multiple human pathologies, including cardiovascular diseases⁵⁹. Importantly, PARP-1 is a viable pharmacological target for vasoprotection^{41,60}.

Role of additional conserved molecular pathways impacting aging processes

During the past two decades common themes have emerged regarding the role of overlapping evolutionarily conserved processes that promote aging. Here we provide a brief overview of the available evidence on the role of some of these pathways in endothelial pathophysiology in aging.

For endothelial biology SIRT1, a NAD-dependent histone deacetylase with potent anti-aging effects⁶¹, is of special importance. SIRT1 expression and activity decrease with age in the vasculature, which likely contributes to endothelial dysfunction⁵. Accordingly, recent studies show that pharmacological activation of SIRT1 significantly improves endothelial function in aged mice⁶². Further, supplementation with nicotinamide mononucleotide (NMN), a key NAD⁺ intermediate, activates SIRT1 and improves endothelial function in the aged vasculature⁶³. SIRT1 and other sirtuins are considered promising drug targets⁶¹ and future studies should evaluate the efficacy of sirtuin activating compounds for cardiovascular protection in older persons.

Caloric restriction is a dietary regimen recognized to delay aging and extend lifespan in laboratory animals^{64–67}. Previous studies demonstrate that caloric restriction exerts significant anti-oxidant and anti-inflammatory vascular effects, preserving endothelial function in aged rodents^{65,68}. Importantly, these endothelial protective effects of caloric restriction also appear to be mediated by activation of SIRT1-dependent pathways^{65,68}. Initial evidence from studies in obese subjects suggests that caloric restriction also confers endothelial protective effects in humans⁶⁹. Future studies should define the circulating factors mediating the vasoprotective effects of caloric restriction and establish the translational potential of targeting caloric restriction-sensitive pathways.

Optimal regulation of cellular energy metabolism is a crucial requirement for healthy aging. AMP-activated kinase (AMPK) is an evolutionarily conserved master regulator of cellular metabolism, which is known to regulate aging processes. Initial evidence suggests that pharmacological activation of AMPK exerts significant endothelial protective effects⁷⁰.

Inhibition of mechanistic target of rapamycin (mTOR) extends lifespan in mice and invertebrate models and reduces/delays the pathogenesis of various age-related

diseases^{71–73}. mTOR is a serine/threonine protein kinase of the PI(3)K-related family that functions as a master regulator of cellular growth and metabolism by sensing nutrient status. The cellular mechanisms by which mTOR exerts its anti-aging effects include the regulation of proteome dynamics, ribosomal biogenesis, and autophagy through mTOR complex 1⁷³. Important for the present review, there is accumulating evidence that rapamycin treatment exerts significant anti-aging vascular effects, improving endothelial function in rodent models of aging⁷⁴ and age-related diseases^{71,75}. There are strong data demonstrating that rapamycin treatment exerts significant cardioprotective effects by reversing age-dependent cardiac proteome remodeling, which mimics the effects of caloric restriction⁷³. Further studies are needed to test whether similar effects underlie rapamycin-mediated vasoprotection.

Several lines of evidence support the concept that unrepaired DNA damage may impact the aging phenotype of various organ systems. Interestingly, recent studies in mice with genomic instability recapitulate age-related endothelial dysfunction, raising the possibility that the DNA damage response may contribute to vascular aging⁷⁶. In aging endothelial cells may acquire a senescent phenotype, likely due to oxidative stress-induced DNA damage and/or mitochondrial dysfunction¹. There is preliminary evidence that endothelial senescence contributes to endothelial dysfunction in aging and pathophysiological conditions associated with accelerated vascular aging^{77–79}. Importantly, multiple possible “senolytic” agents, which selectively eliminate senescent cells, are under investigation to improve health span at old age. There are reports that long-term senolytic treatment (intermittent treatment with Dasatinib + Quercetin via oral gavage) improves endothelial function in mouse models of aging⁷⁷. However, understanding the exact role of DNA damage response and senescence pathways in endothelial pathophysiology will require further investigations.

Autophagy has also been implicated in the regulation of health span and lifespan. It plays multifaceted roles in vascular aging, including recycling of macromolecules to provide nutrients and energy, as a degradative pathway, and as a critical regulator of organellar (particularly mitochondrial) homeostasis. Old mice treated with an autophagy-enhancing agent have improved NO-mediated vasodilation⁸⁰, suggesting that in aged endothelial cells autophagy is impaired, which contributes to endothelial dysfunction. Future studies should further clarify the role of impaired autophagy in vascular aging, in particular, elucidating its role in mitochondrial dysfunction and oxidative stress.

In the aging field epigenetics is an area of intense current research, examining both DNA modifications (e.g., cytosine methylation) and chromatin status. Technological innovation now allows examination of where in the genome alterations in DNA modification or chromatin are occurring to connect epigenetic changes to altered gene expression^{81,82}. With these advances prior hypothesis like that of genomic hypomethylation are being replaced with a new insights into both tissue specific epigenetic alterations with aging as well as common changes at specific locations in the genome^{83,84}. Much research remains both in determining the epigenetic modifications that occur in specific cellular populations such as endothelial cells with aging impact the expression of many key factors (e.g. eNOS) that regulate endothelial function⁸⁵. Causal studies manipulating the epigenome at specific

locations will need to be performed to link these new data to functional effects, though recent findings demonstrate the difficult nature of these studies⁸⁶. Another important area of study is understanding how post-translational modifications to histone tails, including methylation and acetylation regulate endothelial gene expression and function in aging.

Finally, there is growing evidence suggesting that neuroendocrine mechanisms also have an important role in age-related endothelial alterations⁸⁷. In particular, the age-related decline in circulating growth hormone (GH) and, consequently, hepatic production of insulin-like growth factor-1 (IGF-1) appears to contribute significantly to age-related microvascular changes. IGF-1 deficiency promotes the development of endothelial dysfunction, microvascular rarefaction, and atherosclerosis (recently reviewed in⁸⁸). Studies in animal models of GH and IGF-1 deficiency suggest that the GH/IGF-1 axis regulates endothelial redox homeostasis by attenuating mitochondrial ROS production, up-regulating Nrf2, and maintaining cellular antioxidant enzyme levels^{87,89,90}. Taken together, these results point to potential benefits of interventions preventing age-related IGF-1 deficiency and promoting microvascular health for prevention of cardiovascular diseases in the elderly.

IMPAIRED ANGIOGENESIS IN AGING

Age-related alterations in endothelial phenotypes also have a profound impact on the structural integrity of the circulatory system. The dynamic balance between processes of angiogenesis and vascular regression is critical for maintenance of the microvascular network in the heart and in other organs, including the brain. There is strong evidence that advanced aging is associated with a progressive deterioration of microvascular homeostasis, at least in part, due to age-related impairment of angiogenic processes^{1,91–95}.

The mechanisms contributing to age-related impairment of angiogenesis are likely multifaceted (Figure 2). It is thought that endothelial dysfunction and impaired NO bioavailability play a critical role in angiogenic incompetence. In addition, aging also impairs intrinsic endothelial angiogenic processes by altering key cellular signaling pathways governing proliferation, adhesion, migration, extracellular matrix turnover, apoptosis, synthesis and release of growth factors and cytokines, smooth muscle recruitment, and vessel stabilization⁹⁵. Further, aging also alters pro- and anti-angiogenic circulating factors and results in dysregulated availability of promoters and inhibitors of angiogenesis in various tissues.

Consequences of impaired angiogenesis

It has been proposed that impaired angiogenesis contributes to age-related decline in microvascular density^{96,97}, decreased myocardial blood supply, impaired adaptation to hypoxia^{96,98,99} and exacerbated ischemic tissue injury. Impaired angiogenesis also compromises recovery after tissue damage, which likely contributes to the worsened outcomes of myocardial ischemia and infarction, stroke, and peripheral artery disease in older subjects⁹⁵. During the pathogenesis of cardiac hypertrophy, an imbalance between growth of the cardiac muscle and impaired angiogenesis is likely a critical event leading to cardiac failure. Age-related impairment of angiogenic capacity also has important implications for the utility of therapeutic strategies aimed at facilitating angiogenesis (e.g.

via administration of angiogenic growth factors) in older patients with myocardial ischemia or peripheral artery disease.

Age-related impairment of angiogenesis likely plays a key role in microvascular rarefaction associated with aging^{96,97}. In addition to angiogenic incompetence of endothelial cells, removal of angiogenic stimuli, pericyte dysfunction and up-regulation of angiogenesis inhibitors likely contribute to age-related microvascular rarefaction. Microvascular rarefaction has been linked to both impaired tissue perfusion and development of hypertension¹⁰⁰. Despite these advances the impact of vessel loss on network and vessel level hemodynamics remains under-investigated. As microvascular network resistance is influenced by both the number and the connectivity (i.e. architecture) of vessels, the impact of impaired angiogenesis on network hemodynamics requires evaluation of the integrated effects both in the aged heart⁹⁷ and other organs, including the brain⁶⁴.

Role of impaired bioavailability of NO

Perhaps the most striking finding is that aging promotes intrinsic angiogenic incompetence in endothelial cells. For example, primary microvascular endothelial cells derived from aged rodents exhibit impaired angiogenic response to exogenous administration of vascular endothelial growth factor (VEGF) (Figure 3), suggesting that endothelial cells become resistant to inducers of angiogenesis with age¹⁰¹. There is strong evidence that endothelial synthesis of NO mediates pro-angiogenic effects of VEGF¹⁰² and other growth factors. Diminished bioavailability of endothelium-derived NO impairs the dynamic balance between processes of angiogenesis and vascular regression and promotes microvascular rarefaction. For example, microvascular rarefaction develops in the myocardium upon chronic inhibition of NO synthesis¹⁰³ and in eNOS^{-/-} mice¹⁰⁴. Because aging is associated with endothelial dysfunction and significant decrease in NO bioavailability, these factors likely contribute to impairment of angiogenic processes and microvascular rarefaction with old age⁹². Importantly, the impaired angiogenic capacity of aged endothelial cells compromises the clinical response to interventions aimed to stimulate capillarization in the ischemic myocardium in older individuals in clinical trials⁹⁵. In this regard, it is important to note that the vast majority of preclinical trials promoting therapeutic angiogenesis are conducted in young animals, which do not recapitulate age-related endothelial dysfunction and unresponsiveness of aged endothelial cells to inducers of angiogenesis. This discrepancy may explain the failure to translate promising experimental results into clinical benefits⁹⁵. Interventions that restore NO bioavailability have the potential to improve angiogenesis at old age, although this hypothesis needs to be tested in future pre-clinical and clinical studies. For now, interventions that improve endothelial function in aging appear to have beneficial effects on endothelial angiogenic capacity and/or microvascular capillary density (e.g. exercise, treatment with resveratrol¹⁰⁵).

Role of metabolic dysregulation

Aging is associated with impaired cellular bioenergetics, which directly contributes to functional decline of aged organisms. Interestingly, there is growing evidence that cellular pathways regulating bioenergetics also impact angiogenic processes (reviewed recently in¹⁰⁶). Critical bioenergetic pathways that likely contribute to age-related impairment of

angiogenic processes include the NAD⁺ biosynthetic pathway and activation of the NAD⁺-dependent anti-aging master regulator SIRT1¹⁰⁶. Aging decreases cellular NAD⁺ levels, and there is evidence that treatment with the NAD(+) intermediate nicotinamide mononucleotide reverses both age-associated endothelial dysfunction⁶³ and improves endothelial angiogenic processes (Csizsar et al. unpublished observation 2017). Further, SIRT1 ablation in endothelial cells impairs angiogenesis, mimicking the aging phenotype¹⁰⁷, while treatment of aged animals with pharmacological activators of SIRT1 results in increased microvascular density¹⁰⁵. In myocardial microvascular endothelial cells isolated from aged rats, treatment with a pharmacological activator of the metabolic sensor AMP-activated protein kinase (AMPK), which is linked to SIRT1 activation, also improves angiogenic processes¹⁰⁸.

Role of dysregulation of angiomiR expression

The expression of a number of microRNAs (miRNAs) was reported to change in the heart and the vasculature with aging¹⁰⁹. There is strong evidence that specific miRNAs (angiomiRs)^{110–112} and Dicer1 (ribonuclease III, a key enzyme of the miRNA machinery that is responsible for synthesis of mature functional miRNAs) play important roles in regulating angiogenic processes^{112–115}. Expression of Dicer1 is known to change with age in many tissues¹¹⁶, including microvascular endothelial cells¹¹⁷. There are studies suggesting that age-related dysregulation of Dicer1-dependent microRNA expression in endothelial cells contributes to impaired angiogenesis and microvascular rarefaction in aged rodents^{65,117}. Overexpression of Dicer1 in aged rat endothelial cells significantly improves angiogenic processes, whereas in young endothelial cells, downregulation of Dicer1 resulted in impaired angiogenesis, mimicking the aging phenotype¹¹⁷. Experimental studies suggest that among the angiomiRs dysregulated in aging¹¹⁷, an increased expression of mir-125a-5p is causally linked to dysregulation of eNOS and VEGF in mouse endothelial cells¹¹⁸. Further studies are warranted to understand the role of cell-autonomous and non-cell-autonomous mechanisms contributing to age-related changes in miRNA processing and expression in endothelial cells and to link these changes to specific angiogenic pathways.

Potential role of Nrf2 dysfunction

Nrf2 plays a critical role in maintaining the functional integrity of the microvasculature^{33–35,65}. Recent evidence suggests that Nrf2 also regulates angiogenic processes in endothelial cells and that genetic depletion of Nrf2 impairs angiogenic responses induced by VEGF^{119,120}. Aging is associated with Nrf2 dysfunction in endothelial cells^{34,35}, which may be a potential contributing factor underlying age-related impairment of angiogenesis. In that regard it is interesting that treatment with resveratrol, a potent inducer of Nrf2 activation, improves capillarization in the brain of aged rodents¹⁰⁵.

Role of endothelial senescence

Senescent endothelial cells do not proliferate and their ability to form new vascular structures is impaired¹²¹. Indirect evidence for a causal role of endothelial senescence in impaired angiogenesis and microvascular rarefaction is derived from studies on irradiated laboratory animals¹²². Irradiation is a potent inducer of endothelial DNA damage-induced senescence, mediated by activation of a p16(Ink4a) cyclin dependent kinase inhibitor-dependent pathway¹²¹, which significantly impairs endothelial angiogenic potential. Initial

evidence suggests that in many cell types, including endothelial cells, aging is associated with induction of p16(Ink4a)-dependent pathways (Csiszar, unpublished observation 2017)¹²³. Future studies should determine whether elimination of senescent cells could restore angiogenic potential and/or increase microvascular density in preclinical models of aging. Although a cause-and-effect relationship among telomere shortening, endothelial replicative senescence and impaired angiogenesis has not been established, diminished angiogenic response in telomerase deficient mouse models with critically shortened telomeres¹²⁴ as well as studies in endothelial cells with telomerase overexpression¹²⁵ indicate that telomere exhaustion might also contribute to impairment of endothelial angiogenic potential.

Role of endothelial apoptosis

Several lines of evidence suggest that endothelial cell apoptosis has an important regulatory role in the structural remodeling of microvasculature. For example, endothelial apoptosis may contribute to microvascular rarefaction by counteracting cellular proliferation. There is strong evidence that the number of apoptotic endothelial cells both in large arteries and the microvessels significantly increases with advanced aging^{3,20,126,127}. Previous studies suggest that age-related decline of NO bioavailability, up-regulation of TNF α and/or mitochondrial oxidative stress may promote endothelial apoptosis in the aged vasculature^{20,126}.

Role of systemic circulating factors

Age-related changes in circulating factors may also contribute to impaired angiogenesis in aging⁹⁵. Aging-induced changes in pro-angiogenic endocrine factors are of special importance. Such changes include an age-related decline in circulating levels of GH¹²⁸, IGF-1¹²⁹ and estrogens, all of which regulate multiple aspects of angiogenic processes⁹⁵. Studies in animal models of aging and in animals with circulating GH and IGF-1 deficiency suggest that these age-related changes are causally linked to impaired angiogenesis and microvascular rarefaction in diverse vascular beds¹³⁰. Circulating angiogenic factors, including VEGF¹³¹, also decline during aging. However, impaired angiogenesis and remodeling of the microvasculature are more likely due to changes in the local production of angiogenic or antiangiogenic factors and/or changes in endothelial responsiveness to angiogenic stimuli.

Important insights into the regulation of angiogenesis in aging can be derived from studies on moderate caloric restriction. In laboratory rodents, caloric restriction was demonstrated to exert marked microvascular protective effects, improving microvascular density in the brain⁶⁴. Similarly, chronic intermittent fasting was also shown to exert pro-angiogenic effects, increasing capillary density in the border area of the ischemic myocardium in rats⁶⁷. Caloric restriction appears to up-regulate intrinsic angiogenic processes in endothelial cells⁶⁵, and at least part of these pro-angiogenic effects of caloric restriction are mediated by circulating neuroendocrine factors. In support of this concept, previous studies have shown that circulating factors present in the sera of caloric restricted non-human primates (*M. mulatta*) confer significant pro-angiogenic effects in cultured endothelial cells⁶⁶.

The impact of circulating factors on microvascular density in aging was also demonstrated by studies using heterochronic parabiosis, which involves surgically connecting the circulatory system of a young and an aged mouse¹³². Cerebromicrovascular density typically declines with aging⁹⁶, and the aforementioned studies suggest that circulating factors present in young animals can rejuvenate microvascular architecture in aged heterochronic parabionts¹³². The potential anti-aging factors that improve capillarization may include GDF11¹³². Further studies are warranted to determine how capillary density in the heart is affected by blood factors that change with aging.

Role of dysregulation of promoters and inhibitors of angiogenesis

Aging alters the angiogenesis gene expression signature in the heart (Figure 3) and other tissues¹³³, fostering an anti-angiogenic microenvironment. Age-related changes affect expression of both promoters and inhibitors of angiogenesis (e.g. thrombospondins¹³⁴), and the net effect likely promotes an anti-angiogenic phenotype. Aging is associated with down-regulation of VEGF, a major regulator of angiogenesis in many tissues^{135–137}, and/or resistance to the effects of VEGF¹³⁸. The expression of several other pro-angiogenic factors is also impaired in aging, including expression of IGF-1 and PDGF¹³⁹. PACAP is an evolutionarily conserved neuropeptide secreted by endothelial cells, which confers important antiaging effects. Interestingly there are studies showing that in aged endothelial cells, disruption of autocrine PACAP signaling may contribute to impaired angiogenic capacity¹⁴⁰.

Role of impaired endothelial progenitor cell function

Endothelial progenitor cells (EPCs), which can give rise to both endothelial and mural cell types¹⁴¹, are implicated in angiogenesis stimulated by conditions such as coronary artery disease. Although the definition of true endothelial progenitors is still controversial and there are contradicting data whether the total EPC number is altered by aging^{142–144}, there are studies suggesting that function of circulating EPCs is altered at old age^{143,145,146}. The aging-induced impairment of EPC function is likely mediated, at least in part, by an imbalance between factors promoting cell proliferation, migration, and survival and mediators promoting oxidative stress and inducing apoptosis and/or senescence^{146–148}. There is preliminary evidence that aspects of age-related EPC dysfunction may be reversible by anti-aging interventions¹⁴⁹ and/or by regular aerobic exercise¹⁵⁰. Recent preclinical studies provide also evidence that the serum factors derived from young rats exert beneficial effects on EPCs isolated from aged rats¹⁵¹.

Role of impaired pericyte function

Pericytes play a key role in angiogenesis and maintenance of the structural integrity of the microcirculatory network^{152–154}. Pericytes are recruited to endothelial cells via PDGF/PDGF-R β signaling, and, given their regulation of growth factor presentation, capillary diameter, permeability, endothelial cell proliferation and local extracellular matrix density, they represent a novel therapeutic target^{152–154}. Although age-related changes in pericytes in the heart remain under-investigated, there is evidence that in other organs, including the brain^{152,155} and the kidney¹⁵⁶, an age-related loss of pericyte coverage of microvessels contribute to functional and structural impairment of the microcirculatory network. Yet, it should be noted that some of the age-related changes in pericyte phenotype appears to be

tissue or network region specific¹⁵⁷. The apparent knowledge gap, lack of characterization in the heart, and the focus on unstimulated tissue scenarios, highlight the opportunity and need for the comprehensive characterization of pericyte phenotype, morphology and function over the time course of angiogenesis in aged microvascular networks across multiple tissue types.

FUTURE DIRECTIONS

Although significant progress has been achieved in characterizing aging-induced alterations in endothelial function and endothelial angiogenic phenotypes, the specific roles for cell-autonomous (e.g. mitochondrial dysfunction) and non-cell-autonomous mechanisms (e.g. effects mediated by circulating factors) need to be elucidated further. There is reasonable consensus that NAD(P)H oxidase- and mitochondria-derived ROS play critical roles in vascular aging by promoting endothelial dysfunction. Further investigations are warranted to develop treatments with translational potential, which can improve coronary perfusion in older adults by interfering with these pathways, and thereby to prevent or delay the pathogenesis of cardiovascular disease. The concept that evolutionarily conserved molecular pathways control aging processes in mammals raises the question of whether pharmacological or nutritional modulation of these pathways may be effective in improving endothelial function and/or restoring endothelial angiogenic capacity. In the context of angiogenesis, treatment strategies focusing on the delivery of angiogenic agents could be complemented by anti-aging interventions that improve endothelial health. Promising examples include sirtuin activator compounds⁶¹ and treatments that restore cellular energetics and NAD⁺ levels (e.g. PARP-1 inhibitors). Thus, research efforts should persist in these directions to fully elucidate the roles of fundamental aging processes in vascular pathophysiology and their interaction with other risk factors that contribute to the increased cardiovascular morbidity and mortality in older adults.

Acknowledgement

This work was supported by grants from the American Heart Association (to ST), the National Institute on Aging (R01-AG055395, R01-AG047879; R01-AG038747; P30-AG050911; R01AG049821), the National Institute of Neurological Disorders and Stroke (NINDS; R01-NS056218, R01-NS100782), the National Heart, Lung, and Blood Institute (R01-HL111178; R01-HL134778), the Oklahoma Center for the Advancement of Science and Technology and the Presbyterian Health Foundation. The authors acknowledge the support from the NIA-funded Geroscience Training Program in Oklahoma (T32AG052363).

References

1. Ungvari Z, Kaley G, de Cabo R, Sonntag WE & Csiszar A Mechanisms of vascular aging: new perspectives. *J Gerontol A Biol Sci Med Sci* 65, 1028–1041, doi:glq113 [pii] 10.1093/gerona/glq113 (2010). [PubMed: 20576649]
2. Lerman A & Zeiher AM Endothelial function: cardiac events. *Circulation* 111, 363–368, doi: 10.1161/01.CIR.0000153339.27064.14 (2005). [PubMed: 15668353]
3. Asai K et al. Peripheral vascular endothelial dysfunction and apoptosis in old monkeys. *Arterioscler Thromb Vasc Biol* 20, 1493–1499. (2000). [PubMed: 10845863]
4. Csiszar A et al. Aging-induced phenotypic changes and oxidative stress impair coronary arteriolar function. *Circ Res* 90, 1159–1166 (2002). [PubMed: 12065318]
5. Donato AJ et al. SIRT-1 and vascular endothelial dysfunction with ageing in mice and humans. *J Physiol* 589, 4545–4554, doi:10.1113/jphysiol.2011.211219 (2011). [PubMed: 21746786]

6. Li W et al. Premature death and age-related cardiac dysfunction in male eNOS-knockout mice. *J Mol Cell Cardiol* 37, 671–680 (2004). [PubMed: 15350840]
7. Harman D Aging: A Theory Based on Free Radical and Radiation Chemistry. *J Gerontol*, 298–300 (1956). [PubMed: 13332224]
8. Jablonski KL, Seals DR, Eskurza I, Monahan KD & Donato AJ High-Dose Ascorbic Acid Infusion Abolishes Chronic Vasoconstriction and Restores Resting Leg Blood Flow in Healthy Older Men. *J Appl Physiol*, (in press) (2007).
9. Donato AJ et al. Direct evidence of endothelial oxidative stress with aging in humans: relation to impaired endothelium-dependent dilation and upregulation of nuclear factor-kappaB. *Circulation research* 100, 1659–1666 (2007). [PubMed: 17478731]
10. Adler A et al. NAD(P)H oxidase-generated superoxide anion accounts for reduced control of myocardial O₂ consumption by NO in old Fischer 344 rats. *Am J Physiol Heart Circ Physiol* 285, H1015–1022 (2003). [PubMed: 12915388]
11. Sun D et al. Reduced release of nitric oxide to shear stress in mesenteric arteries of aged rats. *Am J Physiol Heart Circ Physiol* 286, H2249–2256 (2004). [PubMed: 14751861]
12. Francia P et al. Deletion of p66shc gene protects against age-related endothelial dysfunction. *Circulation* 110, 2889–2895 (2004). [PubMed: 15505103]
13. Tschudi MR et al. Effect of age on kinetics of nitric oxide release in rat aorta and pulmonary artery. *J Clin Invest* 98, 899–905 (1996). [PubMed: 8770860]
14. Tanabe T et al. Exercise training improves ageing-induced decrease in eNOS expression of the aorta. *Acta Physiol Scand* 178, 3–10 (2003). [PubMed: 12713509]
15. Woodman CR, Price EM & Laughlin MH Aging induces muscle-specific impairment of endothelium-dependent dilation in skeletal muscle feed arteries. *J Appl Physiol* 93, 1685–1690 (2002). [PubMed: 12381754]
16. Matsushita H et al. eNOS activity is reduced in senescent human endothelial cells: Preservation by hTERT immortalization. *Circ Res* 89, 793–798 (2001). [PubMed: 11679409]
17. Hoffmann J et al. Aging enhances the sensitivity of endothelial cells toward apoptotic stimuli: important role of nitric oxide. *Circ Res* 89, 709–715. (2001). [PubMed: 11597994]
18. Sindler AL, Delp MD, Reyes R, Wu G & Muller-Delp JM Effects of aging and exercise training on eNOS uncoupling in skeletal muscle resistance arterioles. *J Physiol*, doi:jphysiol.2009.172221 [pii] 10.1113/jphysiol.2009.172221 (2009).
19. Berkowitz DE et al. Arginase reciprocally regulates nitric oxide synthase activity and contributes to endothelial dysfunction in aging blood vessels. *Circulation* 108, 2000–2006 (2003). [PubMed: 14517171]
20. Csiszar A et al. Vasculoprotective effects of anti-TNFalpha treatment in aging. *The American journal of pathology* 170, 388–698 (2007). [PubMed: 17200210]
21. Flavahan S, Chang F & Flavahan NA Local Renin-Angiotensin System Mediates Endothelial Dilator Dysfunction in Aging Arteries. *Am J Physiol Heart Circ Physiol*, ajpheart.00422.02016, doi:10.1152/ajpheart.00422.2016 ajpheart.00422.2016 [pii] (2016).
22. Dai DF, Rabinovitch PS & Ungvari Z Mitochondria and cardiovascular aging. *Circ Res* 110, 1109–1124, doi:10.1161/CIRCRESAHA.111.246140 110/8/1109 [pii] (2012). [PubMed: 22499901]
23. Tarantini S et al. Treatment with the mitochondrial-targeted antioxidant peptide SS-31 rescues neurovascular coupling responses and cerebrovascular endothelial function and improves cognition in aged mice. *Aging Cell*, doi:10.1111/accel.12731 (2018).
24. Gioscia-Ryan RA et al. Mitochondria-targeted antioxidant (MitoQ) ameliorates age-related arterial endothelial dysfunction in mice. *J Physiol* 592, 2549–2561, doi:10.1113/jphysiol.2013.268680 jphysiol.2013.268680 [pii] (2014). [PubMed: 24665093]
25. Schriener SE et al. Extension of murine life span by overexpression of catalase targeted to mitochondria. *Science* 308, 1909–1911 (2005). [PubMed: 15879174]
26. Wenzel P et al. Manganese superoxide dismutase and aldehyde dehydrogenase deficiency increase mitochondrial oxidative stress and aggravate age-dependent vascular dysfunction. *Cardiovasc Res* 80, 280–289, doi:cvn182 [pii] 10.1093/cvr/cvn182 (2008). [PubMed: 18596060]

27. Oelze M et al. Glutathione peroxidase-1 deficiency potentiates dysregulatory modifications of endothelial nitric oxide synthase and vascular dysfunction in aging. *Hypertension* 63, 390–396, doi:10.1161/HYPERTENSIONAHA.113.01602 (2014). [PubMed: 24296279]
28. Ungvari ZI et al. Dysregulation of mitochondrial biogenesis in vascular endothelial and smooth muscle cells of aged rats. *Am J Physiol Heart Circ Physiol* 294, H2121–2128 (2008). [PubMed: 18326800]
29. van der Loo B et al. Enhanced peroxynitrite formation is associated with vascular aging. *J Exp Med* 192, 1731–1744. (2000). [PubMed: 11120770]
30. Csiszar A et al. Anti-oxidative and anti-inflammatory vasoprotective effects of caloric restriction in aging: role of circulating factors and SIRT1. *Mech Ageing Dev*, doi:S0047–6374(09)00085–2 [pii] 10.1016/j.mad.2009.06.004 (2009).
31. Burns EM, Kruckeberg TW, Comerford LE & Buschmann MT Thinning of capillary walls and declining numbers of endothelial mitochondria in the cerebral cortex of the aging primate, *Macaca nemestrina*. *J Gerontol* 34, 642–650 (1979). [PubMed: 112144]
32. Burns EM, Kruckeberg TW & Gaetano PK Changes with age in cerebral capillary morphology. *Neurobiol Aging* 2, 283–291 (1981). [PubMed: 7335147]
33. Tarantini S et al. Nrf2 deficiency exacerbates obesity-induced oxidative stress, neurovascular dysfunction, blood brain barrier disruption, neuroinflammation, amyloidogenic gene expression and cognitive decline in mice, mimicking the aging phenotype. *J Gerontol A Biol Sci Med Sci*, in press (2017).
34. Ungvari Z et al. Vascular oxidative stress in aging: a homeostatic failure due to dysregulation of Nrf2-mediated antioxidant response *Am J Physiol Heart Circ Physiol* 301, H363–372. (2011). [PubMed: 21602469]
35. Ungvari Z et al. Age-associated vascular oxidative stress, Nrf2 dysfunction and NF- κ B activation in the non-human primate *Macaca mulatta* *J Gerontol A Biol Sci Med Sci* 66, 866–875 (2011). [PubMed: 21622983]
36. Perridon BW, Leuvenink HG, Hillebrands JL, van Goor H & Bos EM The role of hydrogen sulfide in aging and age-related pathologies. *Aging (Albany NY)* 8, 2264–2289, doi:10.18632/aging.101026 (2016). [PubMed: 27683311]
37. Yuan S et al. Hydrogen sulfide metabolism regulates endothelial solute barrier function. *Redox Biol* 9, 157–166, doi:10.1016/j.redox.2016.08.004 (2016). [PubMed: 27552214]
38. Hine C & Mitchell JR Calorie restriction and methionine restriction in control of endogenous hydrogen sulfide production by the transsulfuration pathway. *Exp Gerontol* 68, 26–32, doi: 10.1016/j.exger.2014.12.010 (2015). [PubMed: 25523462]
39. Ungvari ZI et al. Increased mitochondrial H₂O₂ production promotes endothelial NF- κ B activation in aged rat arteries. *Am J Physiol Heart Circ Physiol* 293, H37–47 (2007). [PubMed: 17416599]
40. Sahoo S, Meijles DN & Pagano PJ NADPH oxidases: key modulators in aging and age-related cardiovascular diseases? *Clin Sci (Lond)* 130, 317–335, doi:10.1042/CS20150087 (2016). [PubMed: 26814203]
41. Csiszar A, Wang M, Lakatta EG & Ungvari ZI Inflammation and endothelial dysfunction during aging: role of NF- κ B. *J Appl Physiol* 105, 1333–1341, doi:90470.2008 [pii] 10.1152/jappphysiol.90470.2008 (2008). [PubMed: 18599677]
42. Lee IM & Paffenbarger RS Jr. Physical activity and stroke incidence: the Harvard Alumni Health Study. *Stroke* 29, 2049–2054 (1998). [PubMed: 9756580]
43. Taddei S et al. Physical activity prevents age-related impairment in nitric oxide availability in elderly athletes. *Circulation* 101, 2896–2901 (2000). [PubMed: 10869260]
44. DeSouza CA et al. Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilation in healthy men. *Circulation* 102, 1351–1357 (2000). [PubMed: 10993851]
45. Spier SA, Delp MD, Stallone JN, Dominguez JM, 2nd & Muller-Delp, J. M. Exercise training enhances flow-induced vasodilation in skeletal muscle resistance arteries of aged rats: role of PGI₂ and nitric oxide. *Am J Physiol Heart Circ Physiol* 292, H3119–3127 (2007). [PubMed: 17337602]

46. Trott DW, Gunduz F, Laughlin MH & Woodman CR Exercise training reverses age-related decrements in endothelium-dependent dilation in skeletal muscle feed arteries. *J Appl Physiol* 106, 1925–1934, doi:91232.2008 [pii] 10.1152/jappphysiol.91232.2008 (2009). [PubMed: 19299569]
47. Durrant JR et al. Voluntary wheel running restores endothelial function in conduit arteries of old mice: Direct evidence for reduced oxidative stress, increased superoxide dismutase activity and down-regulation of NADPH oxidase. *J Physiol*, doi:jphysiol.2009.169771 [pii] 10.1113/jphysiol.2009.169771 (2009).
48. Pacher P, Beckman JS & Liaudet L Nitric oxide and peroxynitrite in health and disease. *Physiological reviews* 87, 315–424 (2007). [PubMed: 17237348]
49. Ma L et al. Anti-peroxynitrite treatment ameliorated vasorelaxation of resistance arteries in aging rats: involvement with NO-sGC-cGKs pathway. *PLoS One* 9, e104788, doi:10.1371/journal.pone.0104788 (2014). [PubMed: 25117910]
50. Xu S et al. Detection of sequence-specific tyrosine nitration of manganese SOD and SERCA in cardiovascular disease and aging. *Am J Physiol Heart Circ Physiol* 290, H2220–2227, doi: 01293.2005 [pii] 10.1152/ajpheart.01293.2005 (2006). [PubMed: 16399855]
51. Burkle A, Beneke S & Muiras ML Poly(ADP-ribose)ylation and aging. *Exp Gerontol* 39, 1599–1601 (2004). [PubMed: 15582275]
52. Pacher P et al. A new, potent poly(ADP-ribose) polymerase inhibitor improves cardiac and vascular dysfunction associated with advanced aging. *The Journal of pharmacology and experimental therapeutics* 311, 485–491 (2004). [PubMed: 15213249]
53. Pacher P et al. Endothelial dysfunction in aging animals: the role of poly(ADP-ribose) polymerase activation. *Br J Pharmacol* 135, 1347–1350 (2002). [PubMed: 11906946]
54. Ha HC, Hester LD & Snyder SH Poly(ADP-ribose) polymerase-1 dependence of stress-induced transcription factors and associated gene expression in glia. *Proc Natl Acad Sci U S A* 99, 3270–3275 (2002). [PubMed: 11854472]
55. Carrillo A et al. Transcription regulation of TNF-alpha-early response genes by poly(ADP-ribose) polymerase-1 in murine heart endothelial cells. *Nucleic Acids Res* 32, 757–766 (2004). [PubMed: 14762203]
56. Andreone TL, O'Connor M, Denenberg A, Hake PW & Zingarelli B Poly(ADP-ribose) polymerase-1 regulates activation of activator protein-1 in murine fibroblasts. *J Immunol* 170, 2113–2120 (2003). [PubMed: 12574383]
57. Zingarelli B et al. Absence of poly(ADP-ribose)polymerase-1 alters nuclear factor-kappa B activation and gene expression of apoptosis regulators after reperfusion injury. *Mol Med* 9, 143–153 (2003). [PubMed: 14571322]
58. Hassa PO & Hottiger MO A role of poly (ADP-ribose) polymerase in NF-kappaB transcriptional activation. *Biol Chem* 380, 953–959 (1999). [PubMed: 10494847]
59. Pacher P & Szabo C Role of the peroxynitrite-poly(ADP-ribose) polymerase pathway in human disease. *Am J Pathol* 173, 2–13, doi:10.2353/ajpath.2008.080019 (2008). [PubMed: 18535182]
60. Berger NA et al. Opportunities for the repurposing of PARP inhibitors for the therapy of non-oncological diseases. *Br J Pharmacol* 175, 192–222, doi:10.1111/bph.13748 (2018). [PubMed: 28213892]
61. Baur JA, Ungvari Z, Minor RK, Le Couteur DG & de Cabo R Are sirtuins viable targets for improving healthspan and lifespan? *Nat Rev Drug Discov* 11, 443–461, doi:10.1038/nrd3738 nrd3738 [pii] (2012). [PubMed: 22653216]
62. Gano LB et al. The SIRT1 activator SRT1720 reverses vascular endothelial dysfunction, excessive superoxide production, and inflammation with aging in mice. *Am J Physiol Heart Circ Physiol* 307, H1754–1763, doi:10.1152/ajpheart.00377.2014 (2014). [PubMed: 25326534]
63. de Picciotto NE et al. Nicotinamide mononucleotide supplementation reverses vascular dysfunction and oxidative stress with aging in mice. *Aging Cell* 15, 522–530, doi:10.1111/accel.12461 (2016). [PubMed: 26970090]
64. Lynch CD et al. Effects of moderate caloric restriction on cortical microvascular density and local cerebral blood flow in aged rats. *Neurobiol Aging* 20, 191–200, doi:S0197458099000329 [pii] (1999). [PubMed: 10537028]

65. Csiszar A et al. Caloric restriction confers persistent anti-oxidative, pro-angiogenic, and anti-inflammatory effects and promotes anti-aging miRNA expression profile in cerebromicrovascular endothelial cells of aged rats. *Am J Physiol Heart Circ Physiol* 307, H292–306, doi:10.1152/ajpheart.00307.2014 (2014). [PubMed: 24906921]
66. Csiszar A et al. Circulating factors induced by caloric restriction in the nonhuman primate *Macaca mulatta* activate angiogenic processes in endothelial cells. *J Gerontol A Biol Sci Med Sci* 68, 235–249, doi:10.1093/gerona/gls158 gls158 [pii] (2013). [PubMed: 22904098]
67. Katare RG, Kakinuma Y, Arikawa M, Yamasaki F & Sato T Chronic intermittent fasting improves the survival following large myocardial ischemia by activation of BDNF/VEGF/PI3K signaling pathway. *J Mol Cell Cardiol* 46, 405–412, doi:10.1016/j.yjmcc.2008.10.027 (2009). [PubMed: 19059263]
68. Csiszar A et al. Anti-oxidative and anti-inflammatory vasoprotective effects of caloric restriction in aging: role of circulating factors and SIRT1. *Mech Ageing Dev* 130, 518–527, doi:S0047–6374(09)00085–2 [pii] 10.1016/j.mad.2009.06.004 (2009). [PubMed: 19549533]
69. Pierce GL et al. Weight loss alone improves conduit and resistance artery endothelial function in young and older overweight/obese adults. *Hypertension* 52, 72–79, doi:10.1161/HYPERTENSIONAHA.108.111427 (2008). [PubMed: 18504322]
70. Lesniewski LA, Zigler MC, Durrant JR, Donato AJ & Seals DR Sustained activation of AMPK ameliorates age-associated vascular endothelial dysfunction via a nitric oxide-independent mechanism. *Mech Ageing Dev* 133, 368–371, doi:10.1016/j.mad.2012.03.011 (2012). [PubMed: 22484146]
71. Lin AL et al. Chronic rapamycin restores brain vascular integrity and function through NO synthase activation and improves memory in symptomatic mice modeling Alzheimer’s disease. *J Cereb Blood Flow Metab* 33, 1412–1421, doi:10.1038/jcbfm.2013.82 jcbfm201382 [pii] (2013). [PubMed: 23801246]
72. Harrison DE et al. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature* 460, 392–395, doi:nature08221 [pii] 10.1038/nature08221 (2009). [PubMed: 19587680]
73. Dai DF et al. Altered proteome turnover and remodeling by short-term caloric restriction or rapamycin rejuvenate the aging heart. *Aging Cell* 13, 529–539, doi:10.1111/accel.12203 (2014). [PubMed: 24612461]
74. Lesniewski LA et al. Dietary rapamycin supplementation reverses age-related vascular dysfunction and oxidative stress, while modulating nutrient-sensing, cell cycle, and senescence pathways. *Aging Cell* 16, 17–26, doi:10.1111/accel.12524 (2017). [PubMed: 27660040]
75. Lin AL et al. Rapamycin rescues vascular, metabolic and learning deficits in apolipoprotein E4 transgenic mice with pre-symptomatic Alzheimer’s disease. *J Cereb Blood Flow Metab* 37, 217–226, doi:0271678X15621575 [pii] 10.1177/0271678X15621575 (2017). [PubMed: 26721390]
76. Durik M et al. Nucleotide excision DNA repair is associated with age-related vascular dysfunction. *Circulation* 126, 468–478, doi:10.1161/CIRCULATIONAHA.112.104380 (2012). [PubMed: 22705887]
77. Roos CM et al. Chronic senolytic treatment alleviates established vasomotor dysfunction in aged or atherosclerotic mice. *Aging Cell* 15, 973–977, doi:10.1111/accel.12458 (2016). [PubMed: 26864908]
78. Bhayadia R, Schmidt BM, Melk A & Homme M Senescence-Induced Oxidative Stress Causes Endothelial Dysfunction. *J Gerontol A Biol Sci Med Sci* 71, 161–169, doi:10.1093/gerona/glv008 (2016). [PubMed: 25735595]
79. Ungvari Z et al. Cerebromicrovascular dysfunction predicts cognitive decline and gait abnormalities in a mouse model of whole brain irradiation-induced accelerated brain senescence. *GeroScience* 39, 33–42. (2017). [PubMed: 28299642]
80. LaRocca TJ et al. Translational evidence that impaired autophagy contributes to arterial ageing. *J Physiol* 590, 3305–3316, doi:10.1113/jphysiol.2012.229690 (2012). [PubMed: 22570377]
81. Masser DR et al. Analysis of DNA modifications in aging research. *Geroscience*, doi:10.1007/s11357-018-0005-3 (2018).

82. Benayoun BA, Pollina EA & Brunet A Epigenetic regulation of ageing: linking environmental inputs to genomic stability. *Nat Rev Mol Cell Biol* 16, 593–610, doi:10.1038/nrm4048 (2015). [PubMed: 26373265]
83. Unnikrishnan A et al. Revisiting the genomic hypomethylation hypothesis of aging. *Ann N Y Acad Sci*, doi:10.1111/nyas.13533 (2018).
84. Horvath S DNA methylation age of human tissues and cell types. *Genome Biol* 14, R115, doi: 10.1186/gb-2013-14-10-r115 (2013). [PubMed: 24138928]
85. Man HS, Yan MS, Lee JJ & Marsden PA Epigenetic determinants of cardiovascular gene expression: vascular endothelium. *Epigenomics* 8, 959–979, doi:10.2217/epi-2016-0012 (2016). [PubMed: 27381277]
86. Galonska C et al. Genome-wide tracking of dCas9-methyltransferase footprints. *Nat Commun* 9, 597, doi:10.1038/s41467-017-02708-5 (2018). [PubMed: 29426832]
87. Toth P et al. IGF-1 deficiency impairs neurovascular coupling in mice: implications for cerebrovascular aging. *Aging Cell* 14, 1034–1044, doi:10.1111/acel.12372 (2015). [PubMed: 26172407]
88. Sonntag WE et al. Insulin-like growth factor-1 in CNS and cerebrovascular aging. *Front Aging Neurosci* 5, 27, doi:10.3389/fnagi.2013.00027 (2013). [PubMed: 23847531]
89. Bailey-Downs LC et al. Liver-specific knockdown of IGF-1 decreases vascular oxidative stress resistance by impairing the Nrf2-dependent antioxidant response: A novel model of vascular aging. *J Gerontol Biol Med Sci* 67, 313–329 (2012).
90. Csiszar A et al. Endothelial function and vascular oxidative stress in long-lived GH/IGF-deficient Ames dwarf mice. *Am J Physiol Heart Circ Physiol* 295, H1882–1894, doi:10.1152/ajpheart.412.2008 (2008). [PubMed: 18757483]
91. Tarnawski AS, Pai R, Tanigawa T, Matysiak-Budnik T & Ahluwalia A PTEN silencing reverses aging-related impairment of angiogenesis in microvascular endothelial cells. *Biochem Biophys Res Commun* 394, 291–296, doi:S0006–291X(10)00402-X [pii] 10.1016/j.bbrc.2010.02.161 (2010). [PubMed: 20193662]
92. Bach MH, Sadoun E & Reed MJ Defects in activation of nitric oxide synthases occur during delayed angiogenesis in aging. *Mech Ageing Dev* 126, 467–473, doi:S0047–6374(04)00256–8 [pii] 10.1016/j.mad.2004.10.005 (2005). [PubMed: 15722105]
93. Sadoun E & Reed MJ Impaired angiogenesis in aging is associated with alterations in vessel density, matrix composition, inflammatory response, and growth factor expression. *J Histochem Cytochem* 51, 1119–1130 (2003). [PubMed: 12923237]
94. Ahluwalia A & Tarnawski AS Activation of the metabolic sensor - AMP activated protein kinase reverses impairment of angiogenesis in aging myocardial microvascular endothelial cells. Implications for the aging heart. *J Physiol Pharmacol* 62, 583–587 (2011). [PubMed: 22204807]
95. Lahteenvuo J & Rosenzweig A Effects of aging on angiogenesis. *Circ Res* 110, 1252–1264, doi: 10.1161/CIRCRESAHA.111.246116 (2012). [PubMed: 22539758]
96. Ingraham JP, Forbes ME, Riddle DR & Sonntag WE Aging reduces hypoxia-induced microvascular growth in the rodent hippocampus. *J Gerontol A Biol Sci Med Sci* 63, 12–20, doi: 63/1/12 [pii] (2008). [PubMed: 18245756]
97. Anversa P, Li P, Sonnenblick EH & Olivetti G Effects of aging on quantitative structural properties of coronary vasculature and microvasculature in rats. *Am J Physiol* 267, H1062–1073. (1994). [PubMed: 8092271]
98. Murugesan N, Demarest TG, Madri JA & Pachter JS Brain regional angiogenic potential at the neurovascular unit during normal aging. *Neurobiol Aging*, doi:S0197–4580(11)00374–5 [pii] 10.1016/j.neurobiolaging.2011.09.022 (2011).
99. Benderro GF & Lamanna JC Hypoxia-induced angiogenesis is delayed in aging mouse brain. *Brain Res* 1389, 50–60, doi:S0006–8993(11)00513–0 [pii] 10.1016/j.brainres.2011.03.016 (2011). [PubMed: 21402058]
100. Goligorsky MS Microvascular rarefaction: the decline and fall of blood vessels. *Organogenesis* 6, 1–10 (2010). [PubMed: 20592859]

101. Ungvari Z et al. Aging-Induced Dysregulation of Dicer1-Dependent MicroRNA Expression Impairs Angiogenic Capacity of Rat Cerebromicrovascular Endothelial Cells. *J Gerontol A Biol Sci Med Sci*, doi:glS242 [pii] 10.1093/gerona/gls242 (2012).
102. Ziche M et al. Nitric oxide synthase lies downstream from vascular endothelial growth factor-induced but not basic fibroblast growth factor-induced angiogenesis. *J Clin Invest* 99, 2625–2634, doi:10.1172/JCI119451 (1997). [PubMed: 9169492]
103. Sladek T, Gerova M, Znojil V & Devat L Morphometric characteristics of cardiac hypertrophy induced by long-term inhibition of NO synthase. *Physiol Res* 45, 335–338 (1996). [PubMed: 9085359]
104. Kubis N, Richer C, Domergue V, Giudicelli JF & Levy BI Role of microvascular rarefaction in the increased arterial pressure in mice lacking for the endothelial nitric oxide synthase gene (eNOS3pt^{-/-}). *J Hypertens* 20, 1581–1587 (2002). [PubMed: 12172320]
105. Oomen CA et al. Resveratrol preserves cerebrovascular density and cognitive function in aging mice. *Front Aging Neurosci* 1, 4, doi:10.3389/neuro.24.004.2009 (2009). [PubMed: 20552055]
106. Sawada N & Arany Z Metabolic Regulation of Angiogenesis in Diabetes and Aging. *Physiology (Bethesda)* 32, 290–307, doi:10.1152/physiol.00039.2016 (2017). [PubMed: 28615313]
107. Maizel J et al. Sirtuin 1 ablation in endothelial cells is associated with impaired angiogenesis and diastolic dysfunction. *Am J Physiol Heart Circ Physiol* 307, H1691–1704, doi:10.1152/ajpheart.00281.2014 (2014). [PubMed: 25239805]
108. Ahluwalia A & Tarnawski AS Activation of the metabolic sensor-AMP activated protein kinase reverses impairment of angiogenesis in aging myocardial microvascular endothelial cells. Implications for the aging heart. *J Physiol Pharmacol* 62, 583–587 (2011). [PubMed: 22204807]
109. de Lucia C et al. microRNA in Cardiovascular Aging and Age-Related Cardiovascular Diseases. *Front Med (Lausanne)* 4, 74, doi:10.3389/fmed.2017.00074 (2017). [PubMed: 28660188]
110. Bonauer A et al. MicroRNA-92a controls angiogenesis and functional recovery of ischemic tissues in mice. *Science* 324, 1710–1713, doi:1174381 [pii] 10.1126/science.1174381 (2009). [PubMed: 19460962]
111. Doebele C et al. Members of the microRNA-17–92 cluster exhibit a cell intrinsic anti-angiogenic function in endothelial cells. *Blood*, doi:10.1182/blood-2010-01-264812 [pii] 10.1182/blood-2010-01-264812.
112. Kuehbach A, Urbich C, Zeiher AM & Dimmeler S Role of Dicer and Drosha for endothelial microRNA expression and angiogenesis. *Circ Res* 101, 59–68, doi:CIRCRESAHA.107.153916 [pii] 10.1161/CIRCRESAHA.107.153916 (2007). [PubMed: 17540974]
113. Suarez Y, Fernandez-Hernando C, Pober JS & Sessa WC Dicer dependent microRNAs regulate gene expression and functions in human endothelial cells. *Circ Res* 100, 1164–1173, doi:01.RES.0000265065.26744.17 [pii] 10.1161/01.RES.0000265065.26744.17 (2007). [PubMed: 17379831]
114. Suarez Y et al. Dicer-dependent endothelial microRNAs are necessary for postnatal angiogenesis. *Proc Natl Acad Sci U S A* 105, 14082–14087, doi:0804597105 [pii] 10.1073/pnas.0804597105 (2008). [PubMed: 18779589]
115. Yang WJ et al. Dicer is required for embryonic angiogenesis during mouse development. *J Biol Chem* 280, 9330–9335, doi:M413394200 [pii] 10.1074/jbc.M413394200 (2005). [PubMed: 15613470]
116. Yan Y et al. Dicer expression exhibits a tissue-specific diurnal pattern that is lost during aging and in diabetes. *PLoS One* 8, e80029, doi:10.1371/journal.pone.0080029 (2013). [PubMed: 24244599]
117. Ungvari Z et al. Aging-Induced Dysregulation of Dicer1-Dependent MicroRNA Expression Impairs Angiogenic Capacity of Rat Cerebromicrovascular Endothelial Cells. *J Gerontol A Biol Sci Med Sci* 68, 877–891, doi:glS242 [pii] 10.1093/gerona/gls242 (2013). [PubMed: 23239824]
118. Che P et al. miR-125a-5p impairs endothelial cell angiogenesis in aging mice via RTEF-1 downregulation. *Aging Cell* 13, 926–934, doi:10.1111/accel.12252 (2014). [PubMed: 25059272]
119. Wei Y et al. Nrf2 acts cell-autonomously in endothelium to regulate tip cell formation and vascular branching. *Proc Natl Acad Sci U S A* 110, E3910–3918, doi:10.1073/pnas.1309276110 (2013). [PubMed: 24062466]

120. Valcarcel-Ares MN et al. Disruption of Nrf2 signaling impairs angiogenic capacity of endothelial cells: implications for microvascular aging. *J Gerontol A Biol Sci Med Sci* 67, 821–829, doi: 10.1093/gerona/glr229 glr229 [pii] (2012). [PubMed: 22219515]
121. Ungvari Z et al. Ionizing radiation promotes the acquisition of a senescence-associated secretory phenotype and impairs angiogenic capacity in cerebromicrovascular endothelial cells: role of increased DNA damage and decreased DNA repair capacity in microvascular radiosensitivity. *J Gerontol A Biol Sci Med Sci* 68, 1443–1457, doi:10.1093/gerona/glt057 glt057 [pii] (2013). [PubMed: 23689827]
122. Warrington JP et al. Whole brain radiation-induced vascular cognitive impairment: mechanisms and implications. *J Vasc Res* 50, 445–457, doi:10.1159/000354227 000354227 [pii] (2013). [PubMed: 24107797]
123. Roos CM et al. Transcriptional and phenotypic changes in aorta and aortic valve with aging and MnSOD deficiency in mice. *Am J Physiol Heart Circ Physiol* 305, H1428–1439, doi:10.1152/ajpheart.00735.2012 ajpheart.00735.2012 [pii] (2013). [PubMed: 23997094]
124. Franco S, Segura I, Riese HH & Blasco MA Decreased B16F10 melanoma growth and impaired vascularization in telomerase-deficient mice with critically short telomeres. *Cancer Res* 62, 552–559 (2002). [PubMed: 11809709]
125. Murasawa S et al. Constitutive human telomerase reverse transcriptase expression enhances regenerative properties of endothelial progenitor cells. *Circulation* 106, 1133–1139 (2002). [PubMed: 12196341]
126. Csiszar A, Ungvari Z, Koller A, Edwards JG & Kaley G Proinflammatory phenotype of coronary arteries promotes endothelial apoptosis in aging. *Physiol Genomics* 17, 21–30 (2004). [PubMed: 15020720]
127. Pearson KJ et al. Resveratrol Delays Age-Related Deterioration and Mimics Transcriptional Aspects of Dietary Restriction without Extending Life Span. *Cell Metab* 8, 157–168 (2008). [PubMed: 18599363]
128. Khan AS, Lynch CD, Sane DC, Willingham MC & Sonntag WE Growth hormone increases regional coronary blood flow and capillary density in aged rats. *J Gerontol A Biol Sci Med Sci* 56, B364–371 (2001). [PubMed: 11487595]
129. Tarantini S et al. Circulating IGF-1 deficiency exacerbates hypertension-induced microvascular rarefaction in the mouse hippocampus and retrosplenial cortex: implications for cerebromicrovascular and brain aging. *Age (Dordr)* 38, 273–289, doi:10.1007/s11357-016-9931-0 (2016). [PubMed: 27613724]
130. Sonntag WE, Lynch CD, Cooney PT & Hutchins PM Decreases in cerebral microvasculature with age are associated with the decline in growth hormone and insulin-like growth factor 1. *Endocrinology* 138, 3515–3520 (1997). [PubMed: 9231806]
131. Scheubel RJ et al. Age-dependent depression in circulating endothelial progenitor cells in patients undergoing coronary artery bypass grafting. *J Am Coll Cardiol* 42, 2073–2080 (2003). [PubMed: 14680729]
132. Katsimpardi L et al. Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. *Science* 344, 630–634, doi:10.1126/science.1251141 science.1251141 [pii] (2014). [PubMed: 24797482]
133. Tucsek Z et al. Aging exacerbates obesity-induced cerebromicrovascular rarefaction, neurovascular uncoupling, and cognitive decline in mice. *J Gerontol A Biol Sci Med Sci* 69, 1339–1352, doi:10.1093/gerona/glu080 (2014). [PubMed: 24895269]
134. van Almen GC et al. MicroRNA-18 and microRNA-19 regulate CTGF and TSP-1 expression in age-related heart failure. *Aging Cell* 10, 769–779, doi:10.1111/j.1474-9726.2011.00714.x (2011). [PubMed: 21501375]
135. Wagatsuma A Effect of aging on expression of angiogenesis-related factors in mouse skeletal muscle. *Exp Gerontol* 41, 49–54, doi:10.1016/j.exger.2005.10.003 (2006). [PubMed: 16289925]
136. Ryan NA et al. Lower skeletal muscle capillarization and VEGF expression in aged vs. young men. *J Appl Physiol* (1985) 100, 178–185, doi:10.1152/jappphysiol.00827.2005 (2006). [PubMed: 16166239]

137. Iemitsu M, Maeda S, Jesmin S, Otsuki T & Miyauchi T Exercise training improves aging-induced downregulation of VEGF angiogenic signaling cascade in hearts. *Am J Physiol Heart Circ Physiol* 291, H1290–1298, doi:10.1152/ajpheart.00820.2005 (2006). [PubMed: 16617130]
138. Mieno S et al. Aging is associated with an impaired coronary microvascular response to vascular endothelial growth factor in patients. *J Thorac Cardiovasc Surg* 132, 1348–1355, doi:10.1016/j.jtcvs.2006.08.043 (2006). [PubMed: 17140953]
139. Sarzani R, Arnaldi G, Takasaki I, Brecher P & Chobanian AV Effects of hypertension and aging on platelet-derived growth factor and platelet-derived growth factor receptor expression in rat aorta and heart. *Hypertension* 18, III93–99 (1991). [PubMed: 1657776]
140. Banki E et al. Age-related decline of autocrine pituitary adenylate cyclase-activating polypeptide impairs angiogenic capacity of rat cerebromicrovascular endothelial cells. *J Gerontol A Biol Sci Med Sci* 70, 665–674, doi:10.1093/gerona/glu116 (2015). [PubMed: 25136000]
141. Bearzi C et al. Identification of a coronary vascular progenitor cell in the human heart. *Proc Natl Acad Sci U S A*, doi:0907622106 [pii] 10.1073/pnas.0907622106 (2009).
142. Chang EI et al. Age decreases endothelial progenitor cell recruitment through decreases in hypoxia-inducible factor 1alpha stabilization during ischemia. *Circulation* 116, 2818–2829, doi:CIRCULATIONAHA.107.715847 [pii] 10.1161/CIRCULATIONAHA.107.715847 (2007). [PubMed: 18040029]
143. Heiss C et al. Impaired progenitor cell activity in age-related endothelial dysfunction. *J Am Coll Cardiol* 45, 1441–1448, doi:S0735–1097(05)00357–8 [pii] 10.1016/j.jacc.2004.12.074 (2005). [PubMed: 15862416]
144. Thijssen DH et al. Haematopoietic stem cells and endothelial progenitor cells in healthy men: effect of aging and training. *Aging Cell* 5, 495–503, doi:ACE242 [pii] 10.1111/j.1474-9726.2006.00242.x (2006). [PubMed: 17081158]
145. Keymel S et al. Impaired endothelial progenitor cell function predicts age-dependent carotid intimal thickening. *Basic Res Cardiol* 103, 582–586, doi:10.1007/s00395-008-0742-z (2008). [PubMed: 18704258]
146. He T, Joyner MJ & Katusic ZS Aging decreases expression and activity of glutathione peroxidase-1 in human endothelial progenitor cells. *Microvasc Res* 78, 447–452, doi:10.1016/j.mvr.2009.08.009 (2009). [PubMed: 19733578]
147. Kushner EJ et al. Aging is associated with a proapoptotic endothelial progenitor cell phenotype. *J Vasc Res* 48, 408–414, doi:10.1159/000324837 (2011). [PubMed: 21625174]
148. Turgeon J et al. Protection against vascular aging in Nox2-deficient mice: Impact on endothelial progenitor cells and reparative neovascularization. *Atherosclerosis* 223, 122–129, doi:10.1016/j.atherosclerosis.2012.05.003 (2012). [PubMed: 22658259]
149. Thum T et al. Age-dependent impairment of endothelial progenitor cells is corrected by growth-hormone-mediated increase of insulin-like growth-factor-1. *Circulation research* 100, 434–443 (2007). [PubMed: 17234973]
150. Hoetzer GL et al. Aging, exercise, and endothelial progenitor cell clonogenic and migratory capacity in men. *J Appl Physiol* 102, 847–852, doi:01183.2006 [pii] 10.1152/jappphysiol.01183.2006 (2007). [PubMed: 17158243]
151. Zhu G et al. Young environment reverses the declined activity of aged rat-derived endothelial progenitor cells: involvement of the phosphatidylinositol 3-kinase/Akt signaling pathway. *Ann Vasc Surg* 23, 519–534, doi:S0890–5096(09)00039–9 [pii] 10.1016/j.avsg.2008.11.013 (2009). [PubMed: 19540437]
152. Bell RD et al. Pericytes control key neurovascular functions and neuronal phenotype in the adult brain and during brain aging. *Neuron* 68, 409–427, doi:S0896–6273(10)00824-X [pii] 10.1016/j.neuron.2010.09.043 (2010). [PubMed: 21040844]
153. Kelly-Goss MR, Sweat RS, Stapor PC, Peirce SM & Murfee WL Targeting pericytes for angiogenic therapies. *Microcirculation* 21, 345–357, doi:10.1111/micc.12107 (2014). [PubMed: 24267154]
154. Stapor PC, Sweat RS, Dashti DC, Betancourt AM & Murfee WL Pericyte dynamics during angiogenesis: new insights from new identities. *J Vasc Res* 51, 163–174, doi:10.1159/000362276 (2014). [PubMed: 24853910]

155. Toth P et al. Age-related autoregulatory dysfunction and cerebrovascular injury in mice with angiotensin II-induced hypertension. *J Cereb Blood Flow Metab* 33, 1732–1742, doi:10.1038/jcbfm.2013.143 (2013). [PubMed: 23942363]
156. Stefanska A et al. Interstitial pericytes decrease in aged mouse kidneys. *Aging (Albany NY)* 7, 370–382, doi:10.18632/aging.100756 (2015). [PubMed: 26081073]
157. Hughes S et al. Altered pericyte-endothelial relations in the rat retina during aging: implications for vessel stability. *Neurobiol Aging* 27, 1838–1847, doi:10.1016/j.neurobiolaging.2005.10.021 (2006). [PubMed: 16387390]
158. Yang J et al. Synchronized age-related gene expression changes across multiple tissues in human and the link to complex diseases. *Sci Rep* 5, 15145, doi:10.1038/srep15145 (2015). [PubMed: 26477495]

KEY POINTS:

- Age-related endothelial dysfunction associated with impairment of angiogenic processes and subsequent pathologic remodeling of the microcirculation contribute to compromised tissue perfusion and exacerbates functional decline in older subjects.
- The mechanisms underlying age-related endothelial dysfunction are multifaceted and likely involve increased oxidative/nitrative stress and alterations in conserved molecular pathways impacting common aging processes (e.g. SIRT1, mTOR, IGF-1 signaling, pathways regulating autophagy and cellular senescence).
- The mechanisms contributing to age-related impairment of angiogenesis likely include impaired bioavailability of NO, metabolic dysregulation, altered angiomiR expression, Nrf2 dysfunction, endothelial senescence and apoptosis, alterations in systemic circulating factors modulating endothelial function, impaired pericyte function and dysregulated tissue expression of promoters and inhibitors of angiogenesis.
- Anti-aging interventions that prevent/reverse age-related endothelial dysfunction and improve angiogenesis are expected to confer cardiovascular protection and delay functional decline in older subjects extending health span.

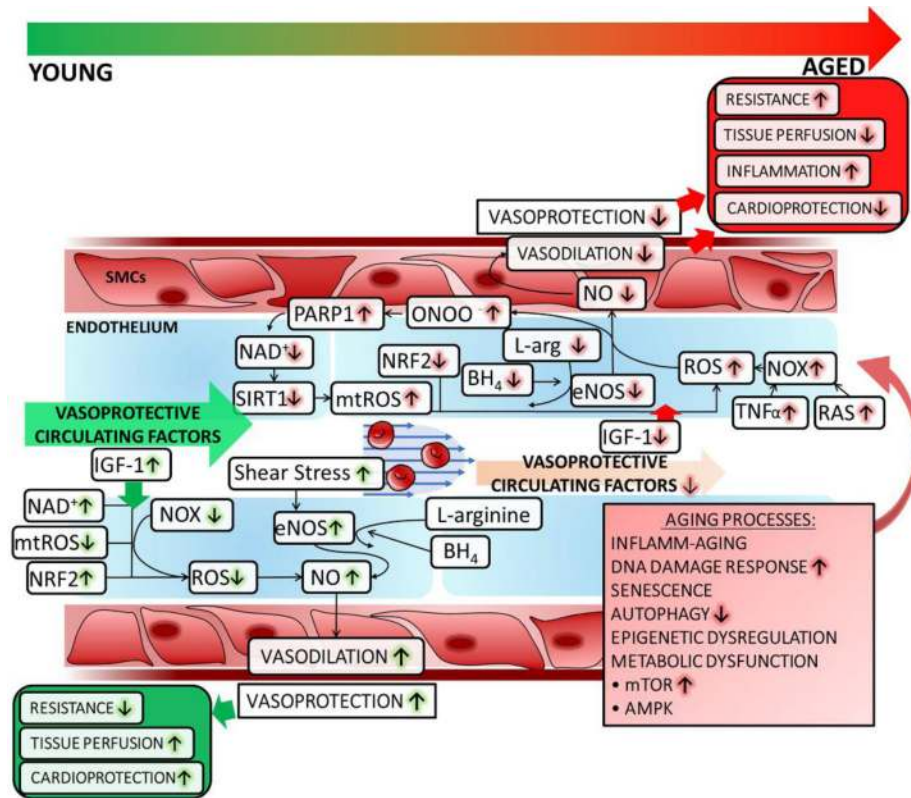


Figure 1: Proposed scheme for aging signaling pathways contributing to oxidative stress and endothelial dysfunction.

In aged endothelial cells increased levels of ROS generated by NOX oxidases (stimulated by elevated TNF α levels and/or by the activated local renin-angiotensin system [RAS] in the vascular wall) and mitochondria decrease the bioavailability of NO by forming ONOO-. Lack of NO leads to vasodilator dysfunction, whereas nitritative stress leads to PARP-1 activation, which contributes to NAD⁺ depletion. Impaired activity of NAD⁺ dependent pro-survival factor SIRT1 and the Nrf2-dependent antioxidant defense pathway exacerbates vascular oxidative stress and endothelial dysfunction in aging. The model predicts that in addition to these cell-autonomous mechanisms age-related deficiency of circulating IGF-1 and other vasoprotective factors confers pro-oxidative vascular effects in aging.

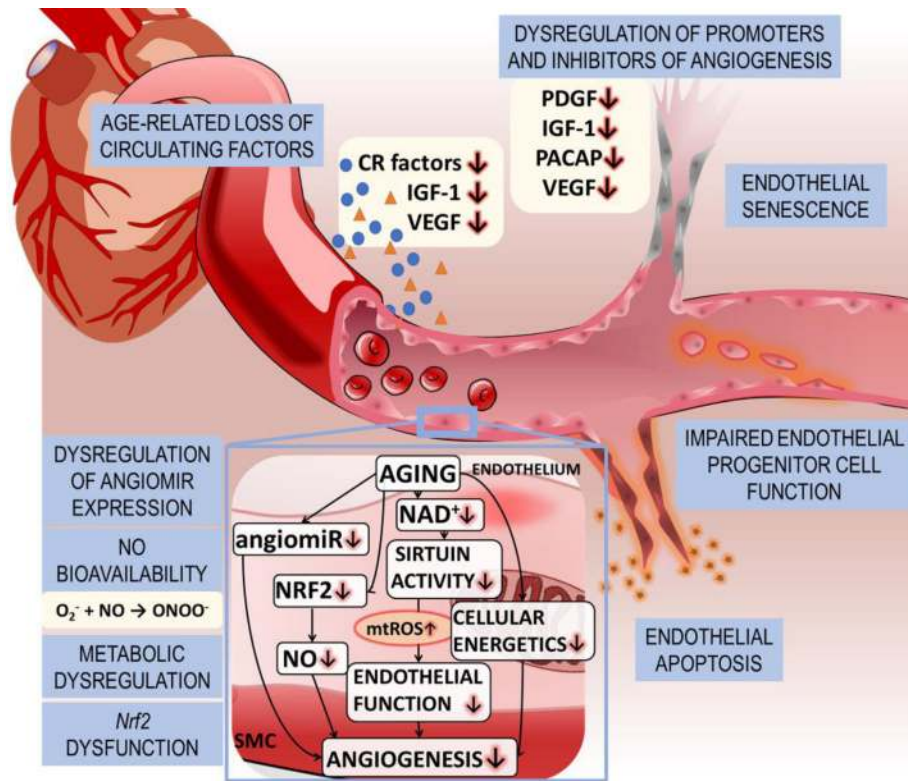


Figure 2: Proposed scheme for the mechanisms by which aging impairs angiogenesis. The proposed model predicts that aging both promotes intrinsic angiogenic incompetence in endothelial cells, rendering them unresponsive to inducers of angiogenesis and, simultaneously, fosters an anti-angiogenic microenvironment in the cardiac tissue.

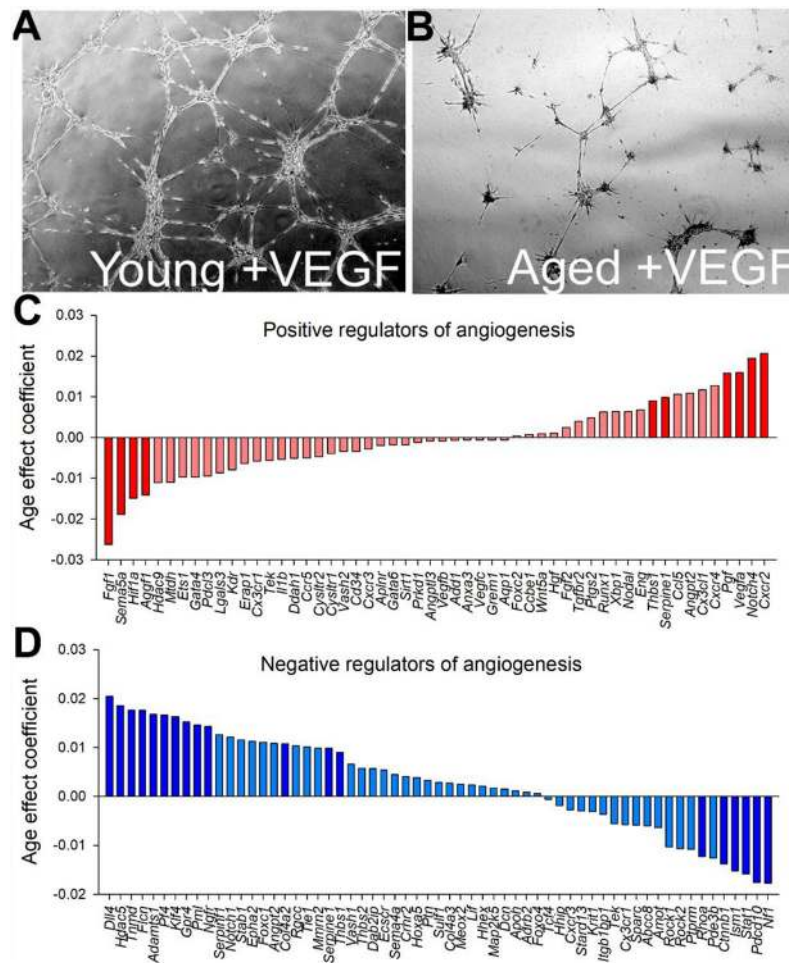


Figure 3: Aging promotes both intrinsic angiogenic incompetence in endothelial cells and dysregulated tissue expression of genes governing angiogenesis.
A-B) Intrinsic tube forming ability of microvascular endothelial cells isolated from aged, 24 month old F344xBN rats (B) is impaired as compared to that of cells isolated from young, 3 month old F344xBN (A). Adapted/Reproduced with permission from¹¹⁷. Endothelial cells were plated on Geltrex matrix-coated wells and formation of capillary-like structures was induced by treating cells with VEGF (100 ng/ml, for 24 h)¹¹⁷. **C-D)** Aging leads to dysregulated expression of both positive- and negative regulators of angiogenesis in the heart. Data are from the published work of Yang et al.¹⁵⁸, filtering for genes of interest (positive regulation of angiogenesis Gene Ontology Term [GO:0045766] and negative regulation of angiogenesis Gene Ontology Term [GO:0016525]) with mouse to human homology and sorted by the magnitude of age effect, based on a log2 fold change per year.