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Endothelial dysfunction and angiogenesis impairment in the ageing vasculature

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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.

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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 countries[1]. Cardiovascular diseases (CVD) is the foremost cause of death and disability among the elderly in the Western world[1]. CVD is a disease of aging; currently approximately 66% of CVDs occur in subjects 75 years of age or more[1]. Age-specific mortality rates from CVD exponentially increase with age throughout the later years of life[1]. 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 in[2]). Clinical and preclinical studies demonstrate that aging is a major cause for endothelial dysfunction[3–5]. 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 metabolism[1]. 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 mortality[6]. 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 Harman[7], 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 animals[4] 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 circulation[4], which is a critical mechanism for maintaining normal myocardial perfusion. The severe generalized impairment of NO bioavailability in aging[13] is also likely exacerbated by an age-related dysregulation of eNOS expression[4,14–17], decreased availability of tetrahydrobiopterin[18] and/or a reduced intracellular L-arginine availability[19].

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 arteries[4]. Previous studies suggest that up-regulation of endothelial NOX in the aged vasculature may be due to the increased presence of pro-inflammatory mediators[20] and/or increased activity of the renin-angiotensin system[21]. 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 system[22]. 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 pathology[25] 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 chain[28], peroxynitrite-mediated nitration and inactivation of manganese superoxide dismutase (MnSOD)[29], decline in glutathione content[30], 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 stress[22]. 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 mechanisms[33]. 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 (H_2S) produced by the evolutionary conserved transsulfuration pathway[36]. Endogenous H_2S exerts vasoprotective effects, promoting vasodilation, regulating endothelial barrier function[37], inducing SIRT1 activation, up-regulating Nrf2 and reducing mitochondrial oxidative stress[36]. Production of H_2S decreases in aging and is increased by anti-aging interventions including calroric restriction and methionine restriction[38].

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-κB 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 exercise[42]. 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 peroxynitrite[48]. 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 remodeling[48]. 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 animals[41]. 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-κB activation[56–58]. The peroxynitrite-PARP-1 pathway has also been implicated in multiple human pathologies, including cardiovascular diseases[59]. 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 effects[61], is of special importance. SIRT1 expression and activity decrease with age in the vasculature, which likely contributes to endothelial dysfunction[5]. Accordingly, recent studies show that pharmacological activation of SIRT1 significantly improves endothelial function in aged mice[62]. Further, supplementation with nicotinamide mononucleotide (NMN), a key NAD+ intermediate, activates SIRT1 and improves endothelial function in the aged vasculature[63]. SIRT1 and other sirtuins are considered promising drug targets[61] 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 humans[69]. 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 effects[70].

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 1[73]. 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 aging[74] 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 restriction[73]. 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 aging[76]. In aging endothelial cells may acquire a senescent phenotype, likely due to oxidative stress-induced DNA damage and/or mitochondrial dysfunction[1]. 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 aging[77]. 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 vasodilation[80], 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 function[85]. 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 studies[86]. 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 alterations[87]. 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 in[88]). 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 stabilization[95]. 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 subjects[95]. 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 hypertension[100]. 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 heart[97] and other organs, including the brain[64].

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 age[101]. There is strong evidence that endothelial synthesis of NO mediates pro-angiogenic effects of VEGF[102] 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 synthesis[103] and in $eNOS^{-/-}$ mice[104]. 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 age[92]. 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 trials[95]. 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 benefits[95]. 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 resveratrol[105]).

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 in[106]). 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 SIRT1[106]. Aging decreases cellular NAD+ levels, and there is evidence that treatment with the NAD(+) intermediate nicotinamide mononucleotide reverses both age-associated endothelial dysfunction[63] and improves endothelial angiogenic processes(Csiszar et al. unpublished observation 2017). Further, SIRT1 ablation in endothelial cells impairs angiogenesis, mimicking the aging phenotype[107], while treatment of aged animals with pharmacological activators of SIRT1 results in increased microvascular density[105]. 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 processes[108].

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 aging[109]. 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 tissues[116], including microvascular endothelial cells[117]. 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 phenotype[117]. Experimental studies suggest that among the angiomiRs dysregulated in aging[117], an increased expression of mir-125a-5p is causally linked to dysregulation of eNOS and VEGF in mouse endothelial cells[118]. 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 rodents[105].

Role of endothelial senescence

Senescent endothelial cells do not proliferate and their ability to form new vascular structures is impaired[121]. Indirect evidence for a causal role of endothelial senescence in impaired angiogenesis and microvascular rarefaction is derived from studies on irradiated laboratory animals[122]. Irradiation is a potent inducer of endothelial DNA damage-induced senescence, mediated by activation of a p16(Ink4a) cyclin dependent kinase inhibitor-dependent pathway[121], which significantly impair 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 telomeres[124] as well as studies in endothelial cells with telomerase overexpression[125] 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 TNFα 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 aging[95]. Aging-induced changes in pro-angiogenic endocrine factors are of special importance. Such changes include an age-related decline in circulating levels of GH[128], IGF-1[129] and estrogens, all of which regulate multiple aspects of angiogenic processes[95]. 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 beds[130]. Circulating angiogenic factors, including VEGF[131], 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 brain[64]. Similarly, chronic intermittent fasting was also shown to exert pro-angiogenic effects, increasing capillary density in the border area of the ischemic myocardium in rats[67]. Caloric restriction appears to up-regulate intrinsic angiogenic processes in endothelial cells[65], 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 cells[66].

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 mouse[132]. Cerebromicrovascular density typically declines with aging[96], and the aforementioned studies suggest that circulating factors present in young animals can rejuvenate microvascular architecture in aged heterochronic parabionts[132]. The potential anti-aging factors that improve capillarization may include GDF11[132]. 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 tissues[133], fostering an anti-angiogenic microenvironment. Age-related changes affect expression of both promoters and inhibitors of angiogenesis (e.g. thrombospondins[134]), 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 VEGF[138]. The expression of several other pro-angiogenic factors is also impaired in aging, including expression of IGF-1 and PDGF[139]. 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 capacity[140].

Role of impaired endothelial progenitor cell function

Endothelial progenitor cells (EPCs), which can give rise to both endothelial and mural cell types[141], 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 interventions[149] and/or by regular aerobic exercise[150]. Recent preclinical studies provide also evidence that the serum factors derived from young rats exert beneficial effects on EPCs isolated from aged rats[151].

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-Rβ 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 kidney[156], 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 specific[157]. 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 compounds[61] 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.

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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.

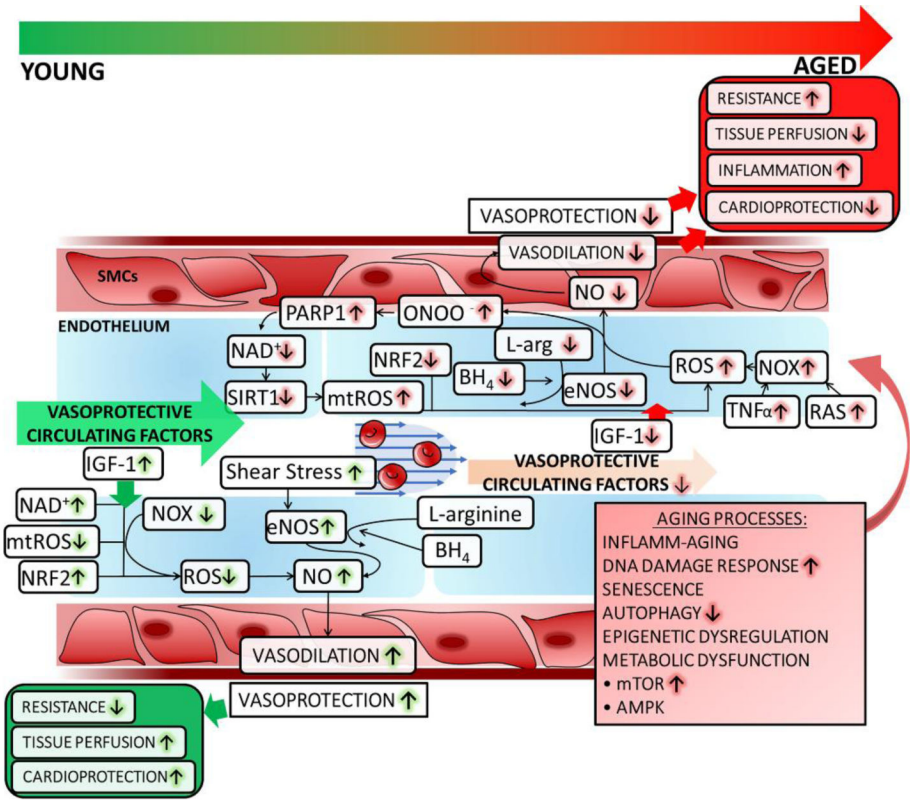

Figure 1: Proposed scheme for aging signaling pathways contributing to oxidative stress and endothelial dysfunction.

In aged endothelial cells increased levels of ROS generated by NOX oxidases (stimulated by elevated TNFα 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 nitrative 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.

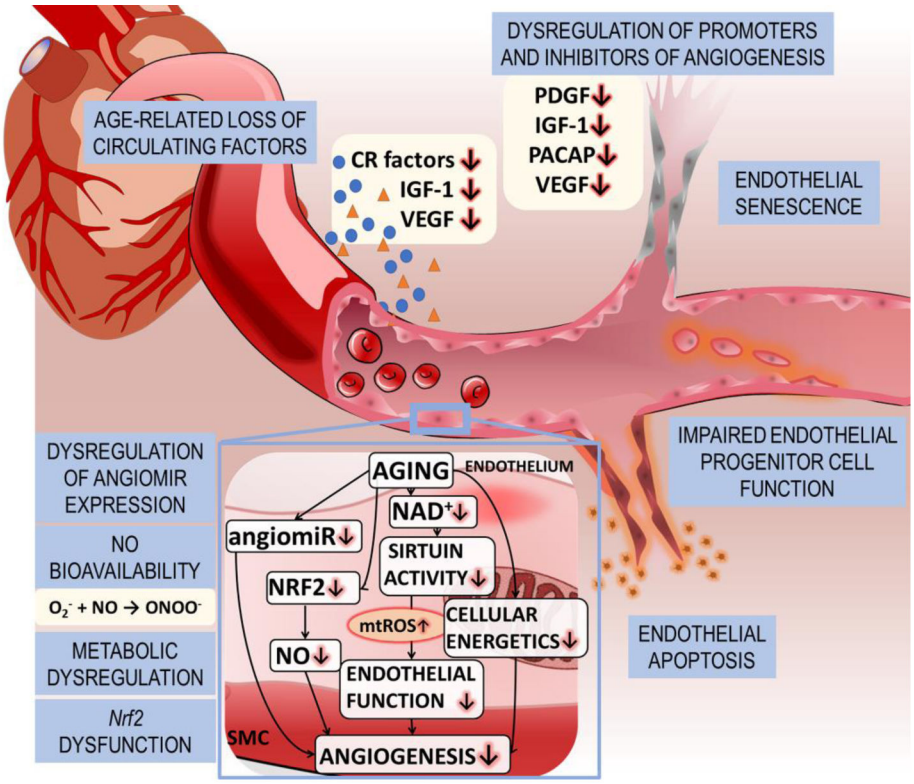

Figure 2: Proposed scheme for the mechanisms by which aging impairs angiogenesis.

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.

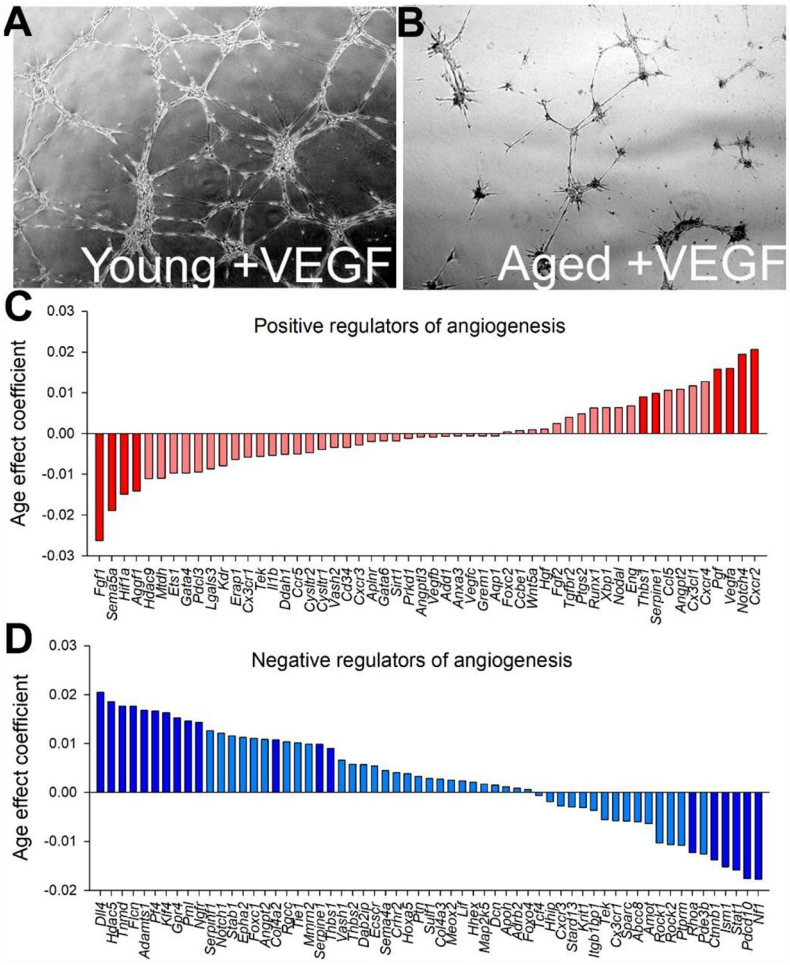

Figure 3: Aging promotes both intrinsic angiogenic incompetence in endothelial cells and dysregulated tissue expression of genes governing angiogenesis.

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 from[117]. 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.