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Mechanisms of Vascular Aging

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Abstract

Aging of the vasculature plays a central role in morbidity and mortality of older people. In order to develop novel treatments for amelioration of unsuccessful vascular aging and prevention of age-related vascular pathologies it is essential to understand the cellular and functional changes that occur in the vasculature during aging. In this review, the pathophysiological roles of fundamental cellular and molecular mechanisms of aging, including oxidative stress, mitochondrial dysfunction, impaired resistance to molecular stressors, chronic low-grade inflammation, genomic instability, cellular senescence, epigenetic alterations, loss of protein homeostasis, deregulated nutrient sensing and stem cell dysfunction in the vascular system are considered in terms of their contribution to the pathogenesis of both micro- and macrovascular diseases associated with old age. The importance of pro-geronic and anti-geronic circulating factors in relation to development of vascular aging phenotypes are discussed. Finally, future directions and opportunities to develop novel interventions to prevent/delay age-related vascular pathologies by targeting fundamental cellular and molecular aging processes are presented.

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Introduction

Cardiovascular and cerebrovascular diseases are the most common cause of death among older people in the United States [1] accounting for approximately 1/3 of all deaths in the US at the age of 65 and nearly 2/3 at an age of 85. With a projected increase in the number of adults over 65 years old increasing from 12 % to 22% in the next 30 years, addressing age related vascular diseases is of critical importance as the annual cost to care for these older people are projected to more than double over that time[2]. As many age-related cardiovascular and cerebrovascular diseases are due to alterations in arterial function or are exacerbated by arterial functional and phenotypic changes, it is important to better elucidate mechanisms underlying arterial aging[3]. Furthermore, as the microcirculation is pervasive, being present in every tissue in the body, it has a unique ability to influence the local environment of the majority of tissues and organs. As such, aging-induced functional and structural alterations of the microcirculation contribute to the pathogenesis of range of age-related diseases including vascular cognitive impairment, Alzheimer's disease, sarcopenia, kidney and eye disease. Therefore, it is also critical to explore the spectrum of age-related microvascular functional and phenotypic changes from subclinical dysfunction to manifested disease as to better predict and prevent microvascular contributions to the pathogenesis of multiple diseases associated with old age. A better mechanistic understanding of macro- and microvascular aging processes is critical to find and evaluate both lifestyle and pharmacological countermeasures to treat this growing health issue.

Rapid advances in geroscience in the last 25 years, including studies on invertebrate models of aging, long-lived mammals, transgenic mouse strains and interventional studies, have led to the identification of evolutionarily conserved pathways involved in lifespan regulation, as well as common denominators of aging in different organisms[4]. In this review, the pathophysiological roles of these aging mechanisms, including oxidative stress, mitochondrial dysfunction, impaired resistance to molecular stressors, chronic low-grade inflammation, genomic instability, telomere attrition and cellular senescence, epigenetic alterations, loss of protein homeostasis ("proteostasis"), deregulated nutrient sensing, stem cell exhaustion, and altered intercellular communication in the vascular system are considered in terms of their contribution to the pathogenesis of both micro- and macrovascular diseases (Figure 1). The interconnectedness between the potential mechanisms of vascular aging and the interaction between the cellular and molecular aging processes and disease-specific pathways are discussed. Likewise, the implications of molecular, cellular and system theories of aging for vascular aging phenotypes are considered. Finally, based on our current mechanistic understanding of vascular aging, potential novel targets for intervention to improve cardiovascular and cerebrovascular health are identified. As such, it is critical to move these potential new therapies forward to reduce the morbidity and mortality associated with cardiovascular and cerebrovascular dysfunction/disease and thereby improving healthspan in an increasingly aged population. We will not

discuss in detail data on the effects of preventive measures already available and in clinical use (including physical exercise, smoking cessation, dietary regimens, inhibitors that disrupt the renin – angiotensin system, statins) on vascular aging phenotypes, as these have been the subject of a recent comprehensive reviews[5–8]. The great deal of phenomenological work has been performed on the effects of aging on specific cell types within the vascular wall and on structural alterations and the hemodynamic consequences that result from arterial stiffening. We refer the interested reader to excellent reviews on these topics[6, 9–11].

Molecular and cellular mechanisms of vascular aging

Role of oxidative and nitrative stress

Since Denham Harman first proposed the free radical theory of aging in the 1950s[12] a large amount of data has been published implicating oxidative stress in vascular aging processes (Figure 2). In particular, there is strong evidence that increased production of reactive oxygen species (ROS) by NAD(P)H oxidases[13–17] and mitochondria[18, 19] contributes to endothelial dysfunction and large elastic artery stiffening with advancing age both in laboratory animals[13, 14, 20–24] and humans [16, 25]. Whilst oxidative stress may influence many facets of vascular function with advancing age via oxidation of critical proteins or induction of redox sensitive transcription factors, one of its most potent effects is inactivation of endothelium-derived nitric oxide (NO). Impaired bioavailability of NO is responsible for age-related reduction in endothelium dependent dilation, enhanced vasoconstriction and dysregulation of tissue perfusion[13, 14, 20, 26–30]. There is strong evidence that endothelial dysfunction caused by increased oxidative stress contributes significantly to both impaired dilation of coronary arteries[13], promoting myocardial ischemia and neurovascular uncoupling, impairing the moment-to-moment adjustment of cerebral blood flow to increased oxygen and nutrient demand that occurs with neuronal activation[31, 32]. In addition to inactivation of NO by ROS, alterations in eNOS activation status, substrate (L-arginine) and cofactor (BH4) availability, increased endothelin-1[26] and/or reduced expression of eNOS [33–35] may also contribute to age-related reduction in NO bioavailability. NO exerts potent anti-inflammatory, anti-thrombotic and anti-leukocyte adhesion effects, thus reduction in NO likely promotes a pro-atherogenic vascular phenotype in aging[36–38].

Many of the results of vascular oxidative stress are mediated via production of the highly reactive oxidant peroxynitrite ($ONOO^-$), the reaction product of NO and superoxide[39, 40] which has been well documented in older endothelial cells and arteries [13, 15, 20, 22]. The mechanisms by which peroxynitrite contributes to vascular aging are multifaceted and include direct cytotoxic effects, adverse effects on mitochondrial function, and activation of inflammatory pathways (Figure 2). In particular, oxidative stress and the consequent activation of redox-sensitive cellular signaling pathways, including NF-kB, are thought to be implicated in the inflammatory process in the aged vasculature. This inflammatory process is characterized by increased endothelial activation[41] and pro-inflammatory changes in the cytokine expression profile of aged vascular cells[18, 19, 42]. Increased vascular oxidative stress has also been linked to activation of matrix metalloproteinases (MMPs) and consequential disruption of the structural integrity of aged arteries, potentially contributing large artery stiffening [43] and the pathogenesis of aortic aneurysms[44]. Furthermore, in the cerebral

circulation aging- and hypertension-dependent activation of the ROS-MMP axis promotes the development of cerebral microhemorrhages[45], which contribute to cognitive decline, geriatric psychiatric syndromes and gait disorders[46]. Importantly, in preclinical models similar antioxidative treatments were reported to prevent large artery stiffening [47], cerebral microhemorrhages [45] and aortic aneurysms[44], providing further evidence for shared pathomechanisms.

Role of mitochondrial dysfunction

Mitochondria play central role in regulation of aging processes[4, 48], including regulation of lifespan[49]. As mammals age, the efficacy of the respiratory chain diminishes, promoting electron leakage and increased ROS production and reducing cellular ATP generation.

Recent studies suggest that mitochondrial ROS production (mtROS) has an important role in age-related vascular dysfunction[18, 50–52]. In the aged vasculature increased mtROS has been attributed to a dysfunctional electron transport chain[53] and is likely exacerbated by peroxynitrite-mediated nitration and inhibition of MnSOD[14], decline in cellular glutathione content[54], down-regulation of p66*Shc*[55], and/or impaired Nrf2-mediated antioxidant defense responses[18, 56, 57]. There is also evidence that aging is associated with impaired mitochondrial biogenesis in endothelial and smooth muscle cells both in conduit arteries[53] and the capillaries[58, 59], which is likely to negatively impact cellular energetics and also may increase mitochondrial ROS production by increasing electron flow through the deficient electron transport chain. Importantly, mtROS can be pharmacologically targeted for vasoprotection. For example, treatment with the mitochondrial antioxidant MitoQ[60], resveratrol[61](which substantially attenuates mtROS production in endothelial and smooth muscle cells[19, 62]) and the potent mitochondria-targeted antioxidative tetrapeptide SS-31[51] has been shown to improve endothelial function in arteries from rodent models of aging. Treatment with SS-31 was also shown to restore endothelium-dependent regulation of cerebral blood flow via neurovascular coupling, improving cognitive function in aged mice[51]. There is evidence that mitochondria-derived H_2O_2 promotes low grade vascular inflammation in aging by inducing NF-κB, activation in endothelial and smooth muscle cells[19, 24] (Figure 2). Recent studies also link increased hypertension-induced mtROS production in aged vascular smooth muscle cells[50] to increased MMP activation in the vascular wall and consequential exacerbation of cerebral microhemorrhages[46]. Another potentially important link between mtROS production and vascular aging is the induction of apoptosis via a Bcl-2 dependent pathway[63].

It is increasingly realized that the functional integrity of the vasculature, including regulation of membrane transport and barrier functions, depends of normal cellular energy metabolism. Thus, dysfunctional mitochondria and impaired mitochondrial energy metabolism can potentially contribute to vascular aging in addition to the effects of increased mitochondrial release of ROS. The mitochondrial DNA (mtDNA) has a very high mutation rate, due to the proximity of mtDNA to sites of ROS production in the mitochondria, the lack of protective histone coverage in the mtDNA, and the limited efficiency of mitochondrial DNA repair mechanisms. There is growing evidence that aging increases mutations and deletions in mtDNA, eroding mitochondrial energy production and contributing to aging processes[48].

mtDNA damage is likely a result of increased mitochondrial oxidative stress[64]. Although aging is associated with significant mitochondrial oxidative stress in the vasculature[18, 19, 24, 50], the role of mtDNA damage is development of vascular aging phenotypes is not well understood. To date, only few studies have focused on the link between mitochondrial heteroplasmy and atherogenesis[65]. Mitochondrial DNA deletions have been detected in human atherosclerotic lesions[65, 66], which may contribute to impaired cellular metabolism in the vascular wall. Recent studies provide direct evidence that mitochondrial mutations play a causal role in atherogenesis in mouse models of the disease[67]. Importantly, transgenic mice ApoE$^{-/-}$ harboring a version of the mitochondrial DNA polymerase (polG) deficient in proof-reading activity accumulate mutations in their mtDNA and exhibit accelerated atherosclerosis, associated with impaired proliferation and apoptosis of VSMCs[67].

The NAD$^+$-dependent pro-survival enzyme SIRT1 modulates mitochondrial function in the vasculature, controlling mitochondrial biogenesis, mtROS production and cellular energy metabolism[42, 62, 68] as well as removal of damaged mitochondria by autophagy[69]. The mitochondrial sirtuin SIRT3 also regulates many key enzymes involved in mitochondrial energy metabolism. NAD+ is a rate-limiting co-substrate for sirtuin enzymes and there is growing evidence that cellular NAD$^+$ availability decreases in aging[70, 71], at least in part, due to overactivation of NAD+ utilizing PARP-1[41]. There is strong evidence that in aged mice enhancing NAD$^+$ biosynthesis (e.g by treatment with nicotinamide mononucleotide, a key NAD$^+$ intermediate[70]) rescues age-related functional alterations in the aorta[72] and cerebral vasculature (Tarantini, Csiszar and Ungvari, unpublished findings, 2017), likely by activating sirtuin mediated pathways and reversing age-related decline in mitochondrial function[73]. Other potential mechanisms contributing to impaired bioenergetics in aged cells include oxidation/nitration of mitochondrial proteins, destabilization of the macromolecular organization of electron transport chain complexes and impaired mitophagy (a mitochondrion-specific form of autophagy). The combination of increased mitochondrial damage and decreased turnover of mitochondria, due to impaired biogenesis and deficient mitophagy, likely contribute to the accumulation of dysfunctional mitochondria in the vascular cells, exacerbating vascular aging processes.

Vascular inflammation in aging

There is strong experimental and clinical evidence that chronic, sterile, low-grade inflammation is a hallmark of the aging process (“inflammaging”[74]). Age-related activation of inflammatory processes play a key role in a wide range of macro- and microvascular pathologies, ranging from atherogenesis and aneurysm formation to microvascular dysfunction, blood-brain barrier disruption and Alzheimer’s pathologies[41] (Figure 3). Previous studies demonstrate that both in aged laboratory rodents and primates there is a pro-inflammatory shift in the gene expression profile of vascular endothelial and smooth muscle cells, including an induction of inflammatory cytokines (e.g. IL-6, IL-1β, TNFα), chemokines, adhesion molecules, iNOS and other pro-inflammatory mediators[13, 24, 33, 61, 75–78]. The resulting pro-inflammatory microenvironment in the vascular wall promotes vascular dysfunction[79, 80], impairs cellular metabolism, increases apoptosis[76, 79] and contributes to the pathogenesis of vascular diseases.

The mechanisms contributing to vascular inflammation in aging are likely multifaceted. There is an important cross-talk between increased oxidative stress and activation of inflammatory processes in the aged vascular wall[41]. First, ROS act as signaling molecules activating pro-inflammatory signaling pathways, including NF-κB[24], which regulate endothelial activation and expression of pro-inflammatory paracrine mediators and promote atherogenesis. Importantly, aged endothelial and smooth muscle cells exhibit significant NF-κB activation[24, 56] and selective inhibition of NF-κB in the vasculature was shown to improve blood flow regulation, decrease systemic inflammation, exert beneficial metabolic effects and extend health span[81]. Second, inflammatory mediators are potent inducers of cellular oxidative stress (e.g. TNFα activates NAD(P)H oxidases[79]).

SIRT1 exerts potent anti-inflammatory effects and decreased SIRT1 activity likely contributes to vascular inflammation in aging[42, 82, 83]. Importantly, pharmacological activators of SIRT1 were shown to attenuate vascular inflammation in aged mice[61, 83].

Endothelial senescence (see below) is associated by a significant increase in the production/release of a wide range of inflammatory cytokines and chemokines[84], termed the "senescence-associated secretory phenotype", or SASP. Induction of SASP is likely mediated by activation of NF-κB, p38MAPK, the DNA damage response pathway and GATA4[85, 86]. There is growing evidence that presence of senescent cells contributes significantly to the heightened inflammatory status of the aged vasculature[87].

Sterile inflammation in the vascular wall is also exacerbated by danger-associated molecular patterns (DAMPs), which activate innate immune system effectors, including toll-like receptors (TLRs) and the NLRP3 inflammasome complex. TLRs are activated by a wide range of DAMP ligands (e.g. molecules released to the extracellular matrix from necrotic cells, breakdown products of the extracellular, matrix and/or bacterial breakdown products leaking through a damaged gut barrier) and control the secretion of a number of pro-inflammatory paracrine mediators (e.g. IL-1, IL-6, IL-8, TNFα). There is evidence that aging induces a proinflammatory phenotype in vascular smooth muscle cells, at least in part, due to activation of TLR4-mediated, MyD88-dependent signaling pathways[78]. Strong data suggest that the canonical Nlrp3 inflammasome contributes to systemic low-grade age-related sterile inflammation in mice[88], but further studies are needed to establish the exact role of this mechanism in age-related vascular pathologies.

There is growing evidence that impaired oxidative stress resilience in aging also exacerbates vascular inflammation induced by cardiovascular risk factors, including obesity, metabolic disease and hypertension[89–91]. The interaction between aging and the effect of environmental inflammatory factors (e.g. particulate exposure[92]) to exacerbate vascular inflammation warrants further studies.

Further studies are needed to differentiate between adaptive and maladaptive inflammatory responses in the aged vasculature and to define the role of chronic low-grade microvascular inflammation in a wide range of functional impairments in multiple organs. The interaction between cell-autonomous mechanism of age-related vascular inflammation and inflammatory processes induced by bacterial breakdown products getting to the circulation

through a leaky intestinal barrier or by chronic infection with viruses that exhibit endothelial tropism in vascular inflammation also has to be further clarified. For example, over 90% of adults 80 years of age or older have persistent human cytomegalovirus (CMV) infection[93]. CMV replicates in the vascular endothelial cells during the entire life of the host following initial infection and severity of CMV infection (antibody titers) was shown to predict increased incidence of frailty and risk of mortality[94].

Maladaptation to molecular stresses: role of Nrf2 dysfunction

Recent progress in geroscience research has identified a critical hallmark of the aging process: impaired ability of aged cells to respond to molecular stresses and return to homeostasis. In young organisms in response to increased generation of ROS in the vascular endothelial and smooth muscle cells adaptive homeostatic mechanisms are invoked that involve activation of Nrf2-driven antioxidant defense pathways[56, 57, 95]. Nrf2 is an evolutionarily conserved redox sensitive transcription factor, which coordinates the antioxidant response, including up-regulation of enzymes that detoxify ROS and repair ROS-induced macromolecular damage[96]. In the young vasculature this adaptive homeostatic response serves to reduce oxidative stress and attenuate cellular and macromolecular damage caused by increased ROS levels. Induction of Nrf2 was also shown to exert potent anti-inflammatory[97] and pro-angiogenic[98] effects. There is strong evidence that aging promotes Nrf2 dysfunction in the vasculature, exacerbating oxidative stress and increasing sensitivity of aged vascular cells to ROS-mediated cellular and molecular damage[56, 57]. This loss of oxidative stress resilience may be a major determinant for the development of age related vascular pathologies[99]. Importantly, anti-aging effects of caloric restriction is associated with induction of Nrf2-mediated pathways[18, 100]. Further studies are warranted to determine how pharmacological activation of Nrf2 exerts anti-aging vasoprotective effects.

Loss of proteostasis in vascular aging

Impaired maintenance of proteostasis is thought to contribute to organismal aging[101]. There is evidence that activity of proteostasis networks and proteome stability determine healthy cardiac aging[102, 103]. It is suspected that disequilibrium between protein synthesis, maintenance, and degradation also compromises vascular health. Indeed, increased presence of misfolded protein aggregates is associated with cardiovascular diseases[104]. Aging impacts multiple components of the proteostasis systems in heart and the vasculature, including chaperones[105], the ubiquitin-proteasome and the lysosome-autophagy system[101]. Chaperones assist the folding, assembly, disassembly and transport of other proteins and play a major role in preventing protein misfolding and aggregation. Many age-related molecular alterations can impact chaperoning activities. For example, aging results in down-regulation of HSP70 in the vascular tissue[105]. Further, mitochondrial dysfunction and the resulting decline in cellular ATP content likely also impairs the function of ATP-dependent chaperones. The processes of autophagy (macroautophagy, microautophagy, and chaperone-mediated autophagy) allow the degradation and recycling of cellular constituents. Recently hypotheses were put forward that dysregulation of autophagic processes, including mitophagy, may be a common pathway promoting vascular aging and development of age-related vascular diseases[106]. Experimental findings showing increased oxidative stress, impaired bioavailability of NO and up-regulation of inflammatory mediators in autophagy-

deficient endothelial cells support this view[107]. Further, pharmacological interventions that stimulate autophagy (e.g. trehalose or spermidine treatment) were reported to reverse aspects of arterial aging[108, 109]. Proteasomes degrade unneeded or damaged proteins by proteolysis. There is evidence that proteasome activity declines in advanced aging[101] and is diminished in atherosclerotic plaques of elderly patients[110] and in hearts of ageing rats[111]. In addition to direct effects on protein degradation the ubiquitin-proteasome system (UPS) is critical for the activation of key regulators of atherogenesis and vascular inflammation[112]. Future studies should identify the role of the UPS and other proteostatic pathways that are impaired in specific vascular disease states associated with aging and elucidate age-related factors, including neuroendocrine factors, that regulate proteostasis machineries in the vascular wall.

Role of genomic instability

Since the original formulation of Somatic Mutation Theory of aging [113], a large amount of data has been published both in support and against a causal role of DNA damage and mutation accumulation in aging[114]. There is evidence that diverse genetic lesions may accumulate within aged cells, including somatic mutations, chromosomal aneuploidies and copy number variations and telomere shortening. Despite these advances the role of genomic instability in vascular aging is not well understood. Hypotheses that predict that genomic instability plays a role in vascular aging, usually focus on the primary role of oxidative stress-induced DNA damage, illustrating how these hypotheses and the oxidative stress hypothesis of aging are interconnected[115]. Importantly, endothelial cells appear to have less efficient DNA repair pathways than many other cell types[84]. In that regard it is interesting that known interventions that cause extensive DNA damage (e.g. whole brain irradiation) result in significant phenotypic and functional alterations in endothelial cells, promoting microvascular rarefaction, impaired vasodilation and pro-inflammatory changes, mimicking several aspects of the aging phenotype[84, 116, 117]. In vascular endothelial cells DNA damage readily triggers replicative senescence (see below) [84], to prevent propagation of damaged DNA. In light of recent developments it seems to be likely that induction of senescence is a major mechanism by which DNA damage contributes to vascular aging[118].

To elucidate the causal role of DNA damage in vascular aging an interesting recent study reported that mouse models with genomic instability resulting from the defective nucleotide excision repair genes (ERCC1, XPD) exhibit aging-like vascular phenotypes, including endothelial dysfunction, increased vascular stiffness, increased presence of senescence cells and hypertension[119]. However, these mouse models also exhibit severe liver, kidney, bone marrow, neurological and/or bone phenotypes, which associate with reduced lifespan[119] and it is unclear how closely these phenotypes indeed mimic aging. Mice with genetic deficiency in the spindle assembly checkpoint protein BubR1, which promotes progressive aneuploidy, were also shown to exhibit aging-like vascular phenotypes, including endothelial dysfunction, increased vascular stiffness, media atrophy and fibrosis[120]. However, this mouse model also exhibits short life span associated with severe functional deficits in multiple organs, including cachectic dwarfism and lordokyphosis. Thus, the relevance of this model to normal aging remains unresolved. It was also proposed that an association exists between single-nucleotide polymorphisms in human DNA repair genes and vascular stiffness[119], yet the mechanistic role of DNA repair pathways in the genesis of age-related

vascular diseases in humans remains to be determined. There are studies reporting that increased oxidative DNA damage[121] and increased expression of multiple biomarkers of DNA double strand breaks[122] are present in atherosclerotic plaques. While accelerating or retarding repair of double strand breaks in transgenic mouse models have lesser effect on atherogenesis, they significantly alter plaque stability[122].

Children with Hutchinson-Gilford progeria syndrome and other laminopathies exhibit accelerated vascular pathologies leading to fatal myocardial infarction or stroke at a very young age[123]. There is evidence implicating DNA damage response induced by genetic nuclear lamina dysfunction in aging-like phenotypic changes in vascular smooth muscle cells[124, 125], however, it remains to be demonstrated that these pathways also contribute to "normal" vascular aging.

Impairment of mechanisms responsible for maintaining the appropriate length and functionality of telomeres is thought to play a role in vascular aging and hypertension by inducing cellular senescence (see below)[87, 126].

Role of cellular senescence

Cellular senescence is a fundamental aging process in which cells, including vascular endothelial and smooth muscle cells, permanently withdraw from the cell cycle in response to a range of endogenous and exogenous stressors (e.g. ROS, dysfunctional telomeres, DNA damage, paracrine signals) and undergo distinctive phenotypic alterations, including profound pro-inflammatory secretome changes[127] (Figure 4). Recent studies demonstrate that elimination of senescent cells expressing p16INK4A extends lifespan and healthspan in mice[128, 129], suggesting that cellular senescence plays a fundamental role in physiological decline associated with aging. The available evidence indicate that endothelial senescence also contributes to endothelial dysfunction in aging and pathophysiological conditions associated with accelerated vascular aging[117, 130–132]. Endothelial senescence has also been implicated in the pathogenesis of heart failure[133]. Replicative senescence may also be potentially important for impairment of regenerative and angiogenic capacity of the vascular endothelium. Studies using mouse models of irradiation-induced, DNA damage mediated senescence[84] demonstrate that induction of cellular senescence in the neurovascular unit associates with significant cerebromicrovascular dysfunction[117] and microvascular rarefaction, mimicking the aging phenotype. Advanced atherosclerotic lesions contain senescent cells and recent studies using genetic and pharmacological approaches to eliminate senescent cells in $Ldlr^{-/-}$mice suggest that senescent cells promote atherogenesis, contributing to plaque instability by up-regulating matrix metalloproteases[134] and/or by exacerbating vascular inflammation[135]. The hypothesis was put forward that pharmacological treatment with senolytic agents to clear senescence cells may exert atheroprotective effects[134]. Importantly, long-term senolytic treatment was also shown to improve endothelial function in mouse models of aging[130]. However, the exact role of different senescence mechanisms in age-related vascular pathophysiology is not well understood. Future studies should address the relationship among acquisition of a senescence-associated secretory phenotype (SASP) in the endothelium and the vascular smooth muscle cells and specific disease processes. There is evidence that the SASP can

also induce paracrine senescence and/or alter the function of neighboring cells and the role of this mechanism in vascular aging should be further evaluated. The possibility of paracrine transmission of senescence from microvascular endothelial cells to parenchymal cells also requires further investigations. It should be noted that many studies assess only senescence associated β-galactosidase activity as a marker of senescent cells. Future studies assessing should use novel molecular markers of senescence and senescence reporter mouse models and analyze senescence-related gene expression in individual cells.

Role of increased apoptosis and necroptosis

Apoptosis is an evolutionarily conserved cell death program that is tightly regulated and executed through the interaction of extrinsic and intrinsic signaling pathways[136]. There is strong evidence that alterations in apoptotic potential contribute to a number of aging phenotypes across species, including the genesis of age-related cardiovascular pathologies. In the vasculature there is an increased presence of apoptotic endothelial cells, which has been attributed to impaired bioavailability of NO, up-regulation of TNFα and/or mitochondrial oxidative stress[61, 76, 79, 137]. Increased apoptotic cell death likely contributes to aging-induced microvascular rarefaction and the pathogenesis of atherosclerotic vascular diseases and aneurysm formation[11].

Necroptosis is a newly identified form of programmed cell death that does not involve caspase activation but critically depends on receptor-interacting serine-threonine kinase 3 and mixed lineage kinase domain-like (MLKL) and is characterized by morphological features of necrosis[138]. Necroptosis plays a role in inflammaging by promoting pro-inflammatory phenotypic changes in tissues due to the release of cell debris (damage-associated molecular patterns, DAMPs)[139]. DAMPs are a major activator of NLRP3 inflammasome, which is an important mechanism involved in low-grade chronic inflammation in aging (see above). Inhibition of necroptosis either genetically, pharmacologically or by dietary means was shown to reduce inflammation in mouse models[138, 139]. Importantly, biomarkers of necroptosis are evident in the atherosclerotic plaques in apolipoprotein E (ApoE)-knockout mice and inhibition of necroptosis pathways reduce atherosclerosis burden and increases the lifespan in these models[138]. Biomarkers of necroptosis are also increased in human aorta aneurysmal tissues and up-regulation of necroptosis pathways promote aorta aneurysm progression in mouse models[140].

Taken together, pathways involved in programmed cell death are promising targets for interventions in multiple aging-related diseases, including cardiovascular protection as well as prevention of cell loss leading to muscle atrophy and neurodegeneration.

Role of epigenetic alterations

A wide range of epigenetic alterations affect the cells during the lifespan, which may modulate vascular aging phenotypes[141]. Epigenetic changes that may contribute to vascular aging processes involve alterations in DNA methylation patterns, posttranslational modification of histones, microRNAs, long noncoding RNAs and chromatin remodeling.

DNA methylation is thought to be a central regulator of genome function. Aging is associated with complex, likely sexually dimorphic alterations in methylation patterns in

many organs, which can be partially reversed by anti-aging interventions (e.g. caloric restriction) [142]. Also, in animal models of aging altered methylation of genes important for vascular function has been observed[143, 144]. There is preliminary evidence that in vascular diseases DNA methylation patterns of cells within the vascular wall are altered[145], however, understanding the pathogenic role of these changes in vascular aging requires further studies.

Understanding how post-translational histone modifications (lysine methylation, acetylation), which were demonstrated to regulate lifespan in lower organisms and to modulate aging phenotypes in mammals, modulate vascular aging processes is an area of intense current research[4]. Histone acetylation is dynamically regulated by histone acetyltransferases and histone deacetylases (HDACs). There is particularly strong data that decreased activity/expression of class III HDACs (the NAD+ utilizing sirtuin family) contributes to vascular aging[42, 72, 82, 83, 146].

DNA- and histone-modifying enzymes act in concert with key epigenetic factors that determine changes in chromatin architecture, regulating lifespan and healthspan in evolutionarily diverse organisms[4]. Such chromatin remodeling factors include the Polycomb group proteins, which can remodel chromatin such that epigenetic silencing of genes takes place. The role of the Polycomb group proteins in regulation of endothelial progenitor cell function has been extensively studied[147]. There is evidence that expression/activity of many of these chromatin remodeling factors are altered in aging[4], yet, their mechanistic role in vascular aging has yet to be elucidated.

The expression of 60% of human protein coding genes is controlled through post-transcriptional repression by microRNAs [miRNAs], a class of small non-coding RNAs. There is a complex interplay between miRNAs and other epigenetic factors, and dysregulation of miRNA expression is an emerging field in age-related epigenetics. In the vasculature miRNAs contribute to the regulation of important biological processes, including angiogenesis[148], atherogenesis[149] and restenosis[150]. There is growing evidence that aging is associated with dysregulation of miRNA expression in vascular endothelial and smooth muscle cells, which likely contributes to age-related impairment of angiogenic processes[151], decreased cellular stress resilience[18] and plaque formation, destabilization and rupture[152]. Importantly, there is also evidence that IGF-1 deficiency during a critical period during early in life results in persistent changes in post-transcriptional miRNA-mediated control of critical target genes for vascular health, which likely contribute to the deleterious late-life cardiovascular effects known to occur with developmental IGF-1 deficiency[153].

Long noncoding RNAs (lncRNAs) are transcripts longer than 200 nucleotides, which regulate multiple aspects of RNA transcription and translation. There is growing evidence that lncRNAs interact with pro-inflammatory signaling pathways and regulate senescence, however, their role on regulation of vascular aging processes is virtually unknown[154]. Interestingly, there is initial evidence linking the expression of the lncRNA Meg3 to age-related impairment of angiogenic capacity of endothelial cells[155].

Further studies are definitely needed to understand the contribution of alteration of epigenetic patterns to the development of various age-related vascular pathologies and to elucidate age-related changes in cellular mechanisms assuring the generation and maintenance of epigenetic patterns (e.g. DNA methyltransferases, histone acetylases and deacetylases, methylases, and demethylases, protein complexes implicated in chromatin remodeling). Epigenetic alterations are reversible, thus efforts should persist to develop epigenome-influencing interventions for prevention/treatment of age-related vascular diseases.

Role of dysregulated nutrient sensing pathways in vascular aging: mTOR, sirtuins, AMPK

Evolutionarily highly conserved cellular energy sensing pathways were reported to regulate fundamental aging processes by controlling cellular responses to nutrient availability and growth signals, including mTOR (mechanistic/mammalian target of rapamycin) signaling[156], adenosine monophosphate protein kinase (AMPK), and sirtuins.

It is now recognized that mTOR acts as a critical molecular regulator of key anabolic processes, controlling biosynthesis of proteins, lipids and nucleic acids. In low nutrient conditions, mTOR activity is reduced, which relieves mTOR-dependent inhibition of autophagy, a major catabolic cellular process. Reduced activity of the mTOR pathway has been reproducibly shown to regulate aging and extend lifespan in invertebrates and in mice[157]. Consistent with the concept that mTOR regulates fundamental molecular processes of aging, there is growing evidence that attenuating mTOR activity inhibits or delays the pathogenesis of age-associated diseases, including Alzheimer's disease[158, 159]. There is growing evidence that mTOR inhibition also confers protective, anti-aging vascular effects, delaying endothelial cell senescence[160, 161] and promoting endothelium-mediated, NO-dependent vasodilation[159, 162–165]. mTOR inhibition also regulates phenotypic switch of vascular smooth muscle cells[166]. Recently it was reported that chronic treatment with the mTOR inhibitor rapamycin reverses age-associated arterial dysfunction, decreasing vascular stiffness and oxidative stress[167]. The potential significance of these findings for human aging stems from the availability of inhibitors for mTOR signaling pathways that are approved for clinical use. There is considerable excitement about the potential of mTOR inhibitors to treat cancer and neurological diseases of aging, and potentially to improve health span in elderly patients. Preliminary preclinical studies suggest that mTOR inhibition may also exert certain beneficial effects on cardiovascular pathologies associated with old age, including stroke[168–171]. More importantly, recent studies[159, 172] identified mTOR as a major regulator of brain vascular damage and dysfunction in Alzheimer's disease models[159, 173–175] and in models of atherosclerosis[176]. In mouse models of Alzheimer's disease mTOR inhibition with rapamycin was shown to reduce Aß vascular pathology and improve cerebral blood flow via an endothelial NO-dependent mechanism[159, 172], significantly improving cognitive outcomes. Thus, while further studies are needed to define the role of mTOR in vascular aging, the available evidence indicates that mTOR has an important role in cerebrovascular dysfunction associated with neurodegenerative diseases.

Sirtuins and AMPK are critical cellular energy sensors that are activated by cellular metabolic factors, such as NAD^+ in the case of sirtuins and the AMP:ATP ratio for AMPK.

These pathways are thought to be activated in times of low nutrient status in order to provide the cell with enhanced stress resistance to prevent cellular loss or derangement. Both pathways been shown to be enhanced in response to longevity promoting interventions (eg calorie restriction) and are known to interact with one another. Activators of both the sirtuins, specifically Sirt-1 and −6, and AMPK have been effective in improving endothelial function, enhancing NO bioavailability, and reducing oxidative stress and inflammation. Sirtuins are evolutionarily conserved NAD+-dependent protein deacetylases and ADP-ribosyltransferases, which regulate multiple pathways involved in the aging process. There is strong evidence that in the vasculature, activation of members of the sirtuin family, particularly Sirt-1, confers multifaceted anti-aging effects[42, 61, 62, 68, 72, 83, 146, 177–180]. Sirt-1 specifically contributes to the vasoprotective effects of calorie restriction[42, 181], augments eNOS activation [146], reduces oxidative stress, exerts anti-inflammatory effects[180], is anti-apoptotic, reduces DNA damage, and promotes telomere stability. Sirt-6 also exerts endothelial protective and anti-atherogenic effects in mice[182, 183]. In addition to regulating redox homeostasis, mitochondrial function, endothelial vasodilation and protecting against apoptosis and senescence, Sirt-1 and −6 have been shown to regulate the DNA damage response in VSMCs[184]. Studies also suggest that Sirt-1 is reduced in human atherosclerosis and that Sirt-1 exerts anti-atherogenic effects in mouse models by protecting against DNA damage[184]. Importantly, the activity of many sirtuins, including Sirt-1 and −6, is dependent on the cellular NAD+ supply, which is known to decline with age.

Due to the multitude of beneficial effects, many sirtuin activators have been utilized to determine if sirtuin activation can reverse the vascular aging phenotype. Resveratrol, a naturally occurring polyphenol, can activate sirtuins and numerous other pathways and has been used extensively to reverse arterial aging [185, 186]. Additionally, more specific and potent Sirt-1 activators, such as SRT1720, have recapitulated the beneficial effects afforded by resveratrol treatment, demonstrating anti-oxidant and anti-inflammatory effects. However, although SRT1720 rescued endothelial function in aged mice, that was the result of enhanced cyclooxegenase vasodilators instead of increased NO bioavailability. Supplementation with nicotinamide mononucleotide (NMN), a key NAD^+ intermediate, has also been shown increase sirtuin activation[187], resulting in reversal of mitochondrial dysfunction in advanced age[70]. In the aged vasculature, 8 weeks of NMN supplementation increases SIRT-1 activity and reverses age-related endothelial dysfunction and oxidative stress[72]. In summary, Sirt-1 and other sirtuins are considered promising drug targets[180] and future studies should evaluate the efficacy other sirtuin activating compounds for cardiovascular protection in older persons.

AMPK signaling is an important energy sensing pathway that is involved in regulation of aging processes, integrating energy balance, metabolism and cellular stress resistance [188, 189]. In lower organisms increased AMPK activity was shown to extend lifespan. Treatment of aged mice with pharmacological activators of AMPK, including metformin or aminoimidazole carboxamide ribonucleotide (AICAR), was also shown to confer significant health benefits. There is currently an aging clinical trial, TAME (targeting aging with metformin), that aims to determine if metformin can delay the onset of age-related diseases in humans[190]. In the context of vascular aging, AMPK was shown to confer vasoprotective effects, augmenting eNOS activation[191–194]. Furthermore, inhibition of NF-κB signaling by

AMPK suppresses inflammatory processes. Importantly, AMPK activity is reduced in the aorta[195] and cerebral arteries [196] of old rodents. Pharmacological activation of AMPK by AICAR was shown to restore endothelium-dependent vasodilation in old mice [195], suggesting that inactivation of arterial AMPK contributes to age-associated endothelial dysfunction. Still, little is known regarding the effects of AMPK activators on other vascular functions and much more research is needed to better understand the similarities and differences between the effects of various AMPK activators.

Role of the renin-angiotensin system in vascular aging

In model organisms (*C. elegans*) reducing the activity of acn-1, a homologue of the angiotensin converting enzyme (ACE), results in significant extension of lifespan suggesting that peptide hormones produced by these enzymes regulate fundamental aging processes[197]. There is initial evidence that the anti-aging effects of pharmacological ACE inhibitors are mediated by pathways that partially overlap with other evolutionarily conserved mechanisms involved in regulation of lifespan (e.g. FOXO signaling[197]). Studies on laboratory rodents extend these findings showing that inhibition of the renin-angiotensin system (RAS) pharmacologically or by genetic means exerts significant anti-aging effects, extending lifespan and reversing age-related phenotypic and functional changes in the aged vasculature[198–200]. There is growing evidence demonstrating that up-regulation of tissue RAS plays a role in vascular aging promoting intimal thickening and remodeling in large conduit arteries of aged animals and elderly human subjects[77, 201–203]. Proof-of-concept is derived from studies demonstrating that infusion of angiotensin II into young rats promotes aging-like changes in the vascular phenotype, promoting carotid media thickening and intima infiltration by vascular smooth muscle cells[203]. Further, pharmacological inhibition of RAS activity can reduce arterial stiffness in aged animals and elderly humans independently of changes in blood pressure[204, 205]. Up-regulation of RAS in the vascular wall likely also promotes chronic low-grade vascular inflammation and oxidative stress, enhancing the vascular response to injury and rendering the aged vascular wall susceptible to atherogenesis[77, 202]. Activation of RAS/increased angiotensin II levels in aging have also been linked to induction of mitochondrial oxidative stress in the vasculature[206], development of cerebral microhemorrhages[45] and disruption of the blood brain barrier[207].

More recently the view has emerged that an extended renin-angiotensin-aldosterone system, including local expression of mineralocorticoids and their receptors in the vasculature, plays a tissue-specific role in regulation of aging processes. It has been well established that circulating aldosterone regulates water and electrolyte homeostasis and thereby controls blood pressure. In addition, aldosterone also promotes structural and functional alterations in the vasculature, including inflammation and pathological remodeling[208]. Recent studies demonstrate that age-associated changes of aldosterone and mineralocorticoid receptor signaling dysregulation occur in vascular smooth muscle cells, which may contribute to age-associated arterial remodeling[208]. These findings encourage further experimentation aiming to better characterize the pathophysiological relevance of aging-induced alterations in the extended renin-angiotensin-aldosterone system and to the efficiency of treatments targeting the extended renin-angiotensin-aldosterone system for cardiovascular protection in older individuals.

Role of ECM remodeling in vascular aging

The ECM is an important contributor to health and longevity. This non-cellular compartment, ubiquitous to all tissues and organs does not only provide essential mechanical scaffolding but mediates highly dynamic bio-mechanical and bio-chemical signals required for tissue homeostasis, morphogenesis and cell differentiation. Studies on model organisms suggest that evolutionarily conserved pathways regulate ECM remodeling during aging and that promotion of ECM youthfulness by anti-aging interventions is an essential signature of longevity assurance[209]. Aging in mammals also result in significant changes in ECM biosynthesis, postsynthetic modifications of ECM components and alterations of cell-matrix interactions, which contribute to the development of a spectrum of age-related pathologies[210].

Age-related alterations of the ECM, including the subendothelial basement membrane, intima, media, adventitia, and interstitial matrix (which constitute more than half of the mass of the vascular tissue), play a fundamental role in impairment of both structural and regulatory homeostasis of the vasculature[211]. With age, the expression of growth factors that regulate ECM biosynthesis is altered[45] and the synthesis of many ECM components (e.g. elastin) declines, which impairs elasticity and resilience of the vascular wall to mechanical damage and rupture induced by bursts in wall-tension due to pulsatile pressure waves[211]. Age-related ECM changes also likely alter vascular mechano-transduction, dysregulating cell responses to alterations in the hemodynamic environment. Additionally, aging and cellular senescence alter the secretory phenotype of vascular endothelial and smooth muscle cells, increasing MMP secretion[45]. This, together with increased MMP activation[211] induced by high ROS levels compromises the structural integrity of the vasculature and promotes pathological remodeling (e.g. in hypertension), resulting in increased likelihood of aneurysm formation and vessel rupture, including the development of cerebral microhemorrhages[45]. The available evidence suggests that many of these age-related ECM alterations are governed by circulating factors and factors produced in the vascular wall, including the extended renin-angiotensin-aldosteron system (see above) and an age-related decline in circulating IGF-1 [212].

Collagen synthesis is also dysregulated with age in the vascular wall likely due to the effects of increased paracrine action of TGF-β[126], which contributes to vascular fibrosis and arterial stiffening[211]. Additional features that contribute to increased arterial stiffness include decreased elastin synthesis, elastin degradation and fragmentation, elastin calcification, alterations in cross-linking of extracellular matrix components (e.g. by increased presence of advanced glycation end-products [AGEs])[211, 213, 214].

The pathophysiological consequences of age-related ECM remodeling and arterial stiffening have been the subject of a recent comprehensive review by AlGhatrif and Lakatta[6]. In brief, as the large conduit arteries stiffen in aging, aortic pulse wave velocity, systolic pressure and pulse pressure significantly increase[215], whereas diastolic pressure decreases. Decreased diastolic pressure leads to a decline in coronary blood flow. Increased systolic pressure promotes left ventricular remodeling, diastolic dysfunction and exacerbates atherogenesis. Due to the dilation of conduit arteries wall tension significantly increases, contributing to the development of aneurysms. In addition to alterations in the biomechanical properties of large

arteries, age-related ECM remodeling likely also affects microvascular transport and barrier functions[216]. Age-related alteration of the ECM structure and composition are also manifested in the wall of veins, contributing to the pathogenesis of varicosities[217].

Role of pro-geronic and anti-geronic circulating factors: lessons learnt from heterochronic parabiosis and caloric restriction

There is growing evidence that non-cell-autonomous mechanisms play a critical role in orchestrating vascular aging processes (Figure 1). Aging-induced alterations in vasoprotective endocrine factors are of particular importance. Such changes include an age-related decline in circulating levels of GH[218], IGF-1[219] and estrogens, all of which regulate multiple aspects of endothelium-dependent vasodilation[220], autoregulation of blood flow[221], vascular structural remodeling, atherogenesis[222] and angiogenic processes[223].

The impact of circulating factors on aging phenotypes was also demonstrated by studies using mice with heterochronic parabiosis, which involves surgically connecting the circulatory system of a young and an aged mouse[224]. Cerebromicrovascular density typically declines with advanced age[225], and there is initial evidence that circulating "anti-geronic" factors (which reverse/ prevent development of aging phenotypes) present in young mice can rejuvenate microvascular network architecture in aged heterochronic parabionts[224]. The anti-geronic circulating factors present in young mice are currently unknown, and the previously proposed role for GDF11[224] remains controversial. Future studies should identify additional anti-geronic factors that might be targeted by interventions to extend vascular health-span. Pro-geronic circulating factors increase with age and impair tissue homeostasis in young animals. There is initial evidence that mediators secreted by senescent cells (e.g. inflammatory cytokines, such as TNFα[79]) may serve as pro-geronic circulating factors. Further studies are warranted to identify additional pro-geronic proteins and determine their impact on atherogenesis, endothelial function, blood brain barrier integrity and microvascular function in aging.

Additional evidence to support a central role of anti-geronic circulating factors governing vascular aging processes is derived from studies on caloric restriction, a dietary regimen, which improves health and slow the aging process in evolutionarily distant organisms[226]. Caloric restriction was shown to promote a youthful endothelial phenotype by up-regulating and activating eNOS in aged animals[226–228] and perhaps humans[229]. A critical role of anti-geronic circulating factors in vasculoprotective phenotypic responses induced by caloric restriction was first indicated by the observations that *in vitro* treatment of cultured aged endothelial cells with sera derived from caloric restricted animals mimics phenotypic effects observed *in vivo* during caloric restriction, promoting anti-inflammatory and pro-angiogenic effects[42, 230]. Treatment with sera derived from caloric restricted animals up-regulates SIRT-1[231], however, the exact nature of the circulating factor responsible for this effect remains elusive. One potential candidate anti-geronic protein mediating the vasoprotective effects of caloric restriction is adiponectin, whose serum level is known to be increased by caloric restriction[232–234].

Human studies are needed to identify novel pro-geronic and anti-geronic circulating factors and their cofactors, activators or inhibitors/antagonists and to seek associations with vascular

aging phenotypes. Future studies should also identify cellular origins of circulating pro-geronic and anti-geronic factors that impact vascular aging and characterize pathological conditions that alter their levels in circulation with aging. Further, mechanistic studies describing the cellular effects of pro-geronic and anti-geronic circulating factors in the vascular wall are warranted.

Progenitor cell exhaustion

On the basis of the stem cell theory of aging it is postulated that the inability of various types of vascular progenitor cells to continue to replenish the circulatory system with functional differentiated endothelial and smooth muscle cells compromises the biological functions of the aged vasculature. Importantly, aging impairs neovascularization, which depends on the function of highly proliferative endothelial progenitor cells (EPCs). Previous studies provide evidence that aging compromises the function of circulating EPCs [235–237], likely by altering the production of factors promoting cell proliferation, migration, and survival (e.g. IGF-1), and/or by enhancing inflammation and oxidative stress, activating the renin-angiotensin system and promoting senescence. There are data demonstrating that sera derived from young animals improves the function of cultured EPCs isolated from aged rats [238], suggesting a key role for circulating factors. There are also preclinical data extant suggesting that progressive progenitor cell deficits may contribute to the development of atherosclerosis[237]. In particular, treatment with bone marrow–derived progenitor cells isolated from young mice prevents atherosclerosis progression in ApoE−/− mice, whereas treatment with progenitor cells from older mice is ineffective[237]. It should be noted that role of EPCs in the genesis of vascular aging phenotypes in humans is note well understood. For example, patients with peripheral artery disease exhibit low EPC numbers[239], while patients with chronic myocardial ischemia from coronary microvascular disease[240] or abdominal aorta aneurism have significantly higher levels of circulating progenitor cells than age-matched controls[241]. Future studies should investigate the synergistic effects of aging and associated cardiovascular risk factors in humans and determine how declining EPC function contributes to different vascular aging phenotypes.

Future directions

Although significant progress has been achieved in characterizing aging-induced changes in vascular function and phenotype, research efforts should persist in this direction to develop innovative strategies based on recent achievements in the biology of aging to improve vascular health-span. Understanding the interaction of processes of aging and chronic diseases and should be a high priority. Better alignment of preclinical studies on vascular aging and human investigations is needed. Limitations of translating the results of pre-clinical studies should be recognized. An important recent example is caloric restriction[242]. While caloric restriction confers significant lifespan extension and cardiovascular protection in laboratory rodents[5, 18, 42, 100, 226, 243, 244] and in certain cohorts of non-human primates[230, 245], its protective effects in non-human primates in other studies[246] and in patients with multiple cardiovascular risk factors are less evident[247]. Additionally, in cross-sectional studies the older groups may represent a selected long-lived subset of the younger population. There are existing longitudinal studies in humans (e.g. InCHIANTI study) and

non-human primates, and important information related to mechanisms of vascular aging could be derived from add-on studies to these existing cohorts.

Critical areas of vascular aging research include the role of senescence, epigenetics, stress resilience, inflammation, macromolecular damage, proteostasis, mitochondrial and metabolic dysfunction and impaired stem cell biology. The specific roles for cell-autonomous and non-cell-autonomous mechanisms contributing to vascular aging need to be elucidated further. The role of signal transduction pathways linked to regulation of cellular energetics in the vascular aging process should be better defined. Future studies should also lead to improved understanding of the role circadian clocks to vascular aging. New studies investigating cellular heterogeneity in vascular aging are warranted. Stochastic macromolecular damage leads to regional variability in the presence of senescent cells, cells with altered metabolism, mitochondrial dysfunction and increased ROS production. Such regional variability likely contributes to the focal development of vascular pathologies, ranging from atherosclerotic plaques to microhemorrhages. Single-cell gene expression analysis should facilitate better understanding of the pathophysiological role of functional heterogeneity. Finally, the impact of environmental factors and lifestyle choices impact the vascular aging processes should be better understood.

The disposable soma theory of aging predicts age-related functional decline occurs due to the accumulation of random macromolecular damage and that the proportion of energy investment into cellular maintenance and repair processes will determine longevity of the organism. Numerous studies investigating cellular and molecular stress responses in the vasculature agree with the predictions of this theory. For example, cells of the long-lived muroid rodent species white-footed mouse (*Peromyscus leucopus*; maximum lifespan: >8 years) exhibits decreased production of ROS, improved antioxidant defense mechanisms, increased resistance against oxidative stressors, superior DNA repair mechanisms and more efficient mitochondria than the shorter-lived *Mus musculus*[248–250]. Similar findings were reported in the vasculature of the longest-living rodent species, the naked mole-rat (*Heterocephalus glaber*; maximum lifespan: >30 years)[251]. Importantly virtually all of the mechanistic hypotheses related to vascular aging are tested solely in *Mus musculus* (one of the least successfully aging species), which is potentially a source for significant bias and limit the translatability of the results to the clinical scenario. Therefore, there is a great need for interspecies comparative studies using animals with disparate longevity as well as human studies to understand generalized mechanisms of vascular aging and identify translationally relevant treatments for the promotion of vascular health in older adults.

The same cellular and molecular aging processes that affect arterial vessels and capillaries also affect veins and the lymphatic/glymphatic system, likely contributing to various disease pathologies. Examples include the potential role of cerebral venules in neuroinflammation, Alzheimer's disease and cerebral microhemorrhages[46] and the potential link between age-related glymphatic dysfunction and amyloid pathologies[252]. These areas are attractive targets for future studies.

Taken together, instead of targeting a single disease, interventions that target fundamental aging processes have the potential to prevent/delay a range of vascular pathologies and other

age-related diseases simultaneously. In recent years a variety of candidate drugs/interventions have emerged from basic research and translational studies that may target aging processes. These interventions can be adapted for prevention/treatment of age-related vascular pathologies.

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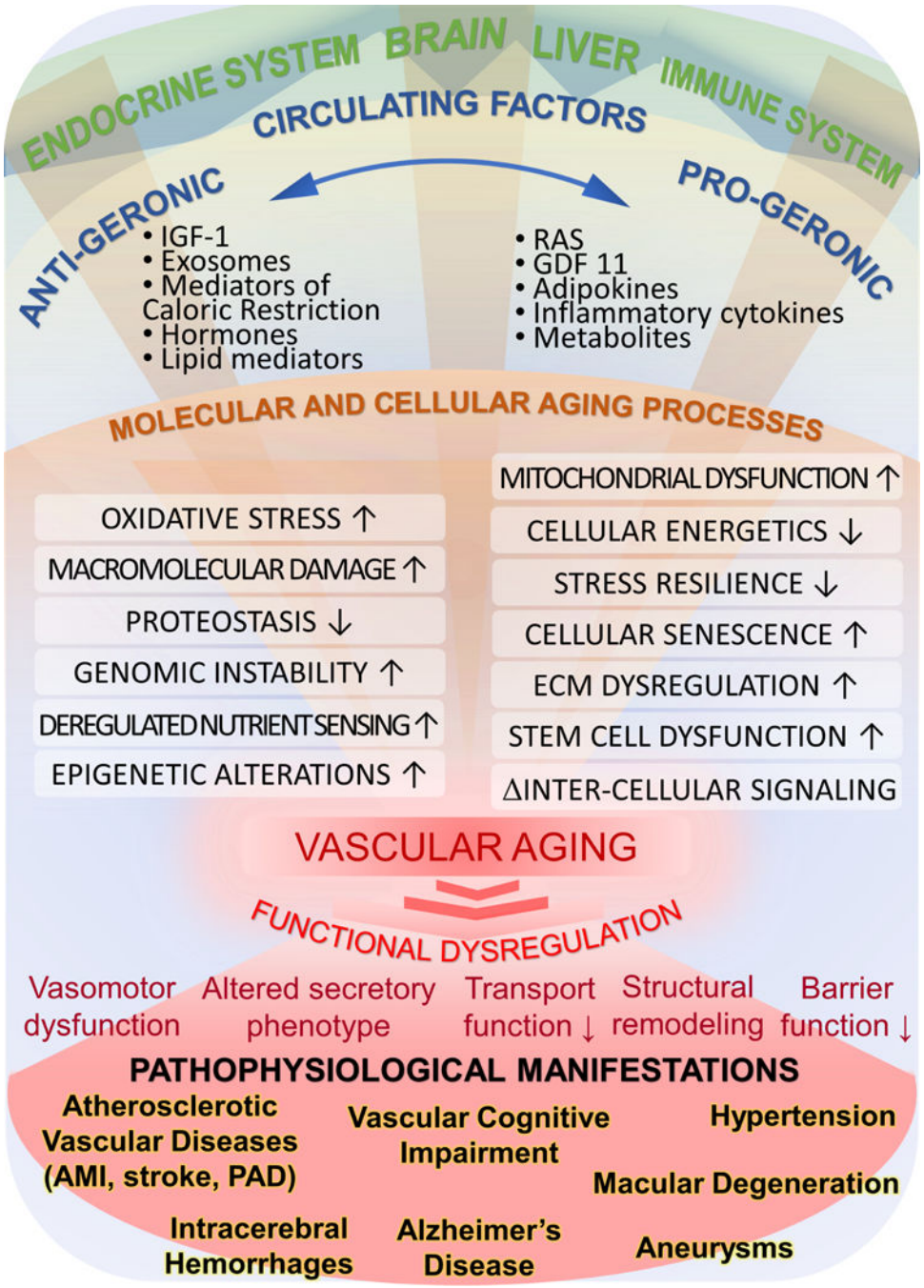

Figure 1: Conceptual model for the role of cell-autonomous and non-cell-autonomous mechanisms in vascular aging.

The model predicts that circulating pro-geronic (e.g. inflammatory cytokines, RAS/renin-angiotensin system, aldosterone) and anti-geronic factors (e.g. IGF-1, mediators of caloric restriction, estrogen) derived from the brain, the endocrine system, cells of the immune system and/or the adipose tissue orchestrate aging processes simultaneously in the endothelial and smooth muscle cells within the large vessels and microcirculation. The hierarchical regulatory cascade for vascular aging involves modulation of cell-autonomous cellular and molecular aging processes. The resulting functional dysregulation of vascular

cells (i.e. impaired vasomotor, barrier, secretory and transport functions of the vasculature as well as adverse structural remodeling) promote the development of a wide range of age-related vascular pathologies.

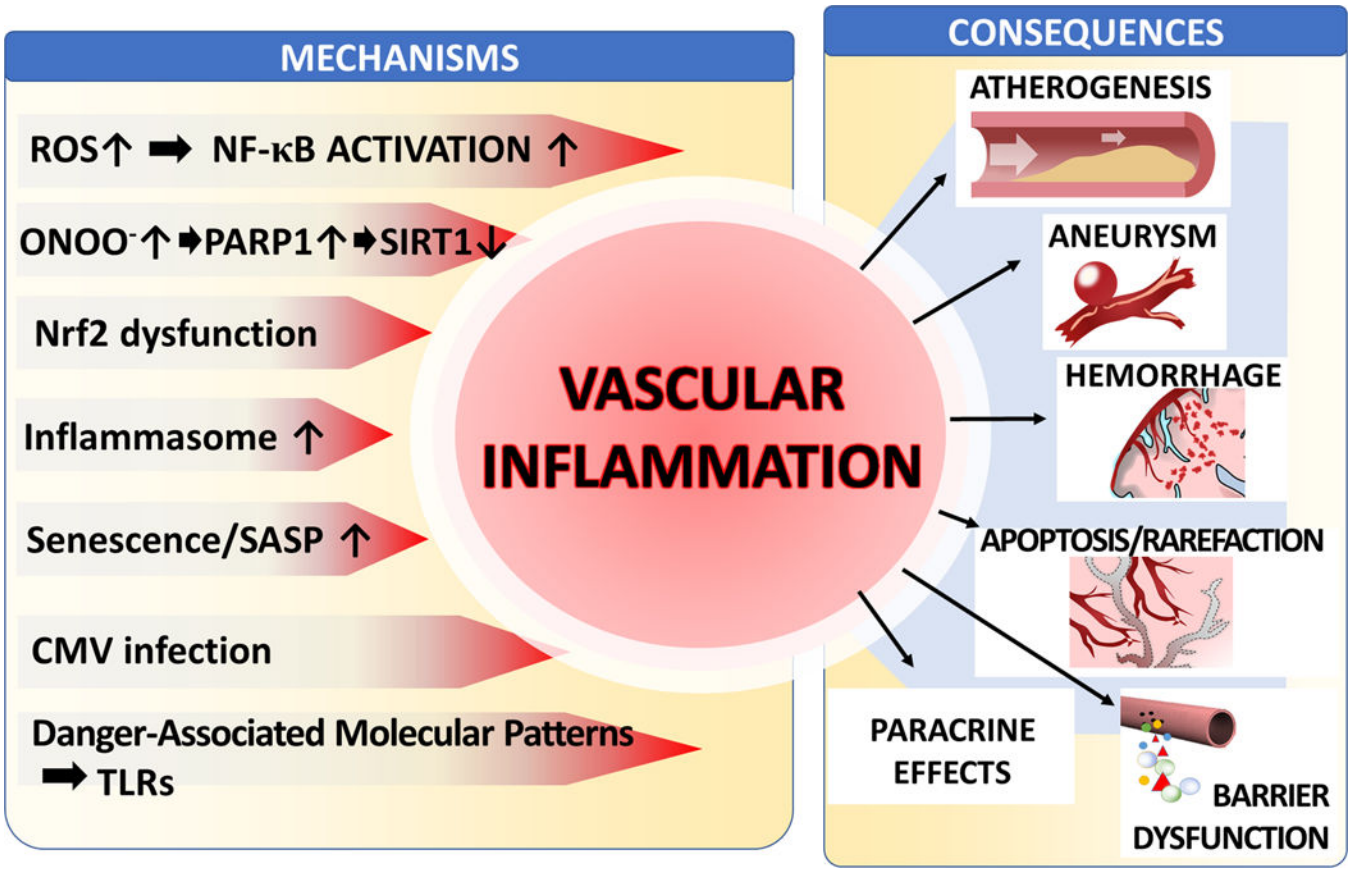

Figure 2: Proposed scheme for mechanisms and pathological consequences of age-related oxidative stress in vascular endothelial cells.

The model predicts that in aged endothelial cells dysfunctional mitochondria and NAD(P)H oxidases are critical sources of increased ROS production. Increased levels of $O_2^{\cdot -}$ generated by the electron transport chain are dismutated to H_2O_2, which can penetrate the mitochondrial membrane increasing cytoplasmic H_2O_2 levels. Increased oxidative stress is exacerbated by age-related impairment of Nrf2-dependent homeostatic antioxidant defense mechanisms. H_2O_2 plays important signaling roles, including activation of NF-κB, which contribute to age-associated low grade chronic vascular inflammation. Increased levels of $O_2^{\cdot -}$ generated by NAD(P)H oxidases (stimulated by elevated TNFα levels and/or by the activated local renin-angiotensin system [RAS] in the vascular wall) decrease the bioavailability of NO by forming ONOO-. Increased nitrative stress lead to PARP-1 activation, which promotes vascular inflammation and contributes to cellular energetic dysfunction by consuming NAD+ , compromising sirtuin-mediated anti-aging pathways. Impaired bioavailability of NO promotes vasodilator dysfunction and compromises endothelial viability. In addition, increased vascular oxidative stress in aging also induces MMP activation, promoting the pathogenesis of intracerebral hemorrhages, aneurysm formation and blood brain barrier disruption.

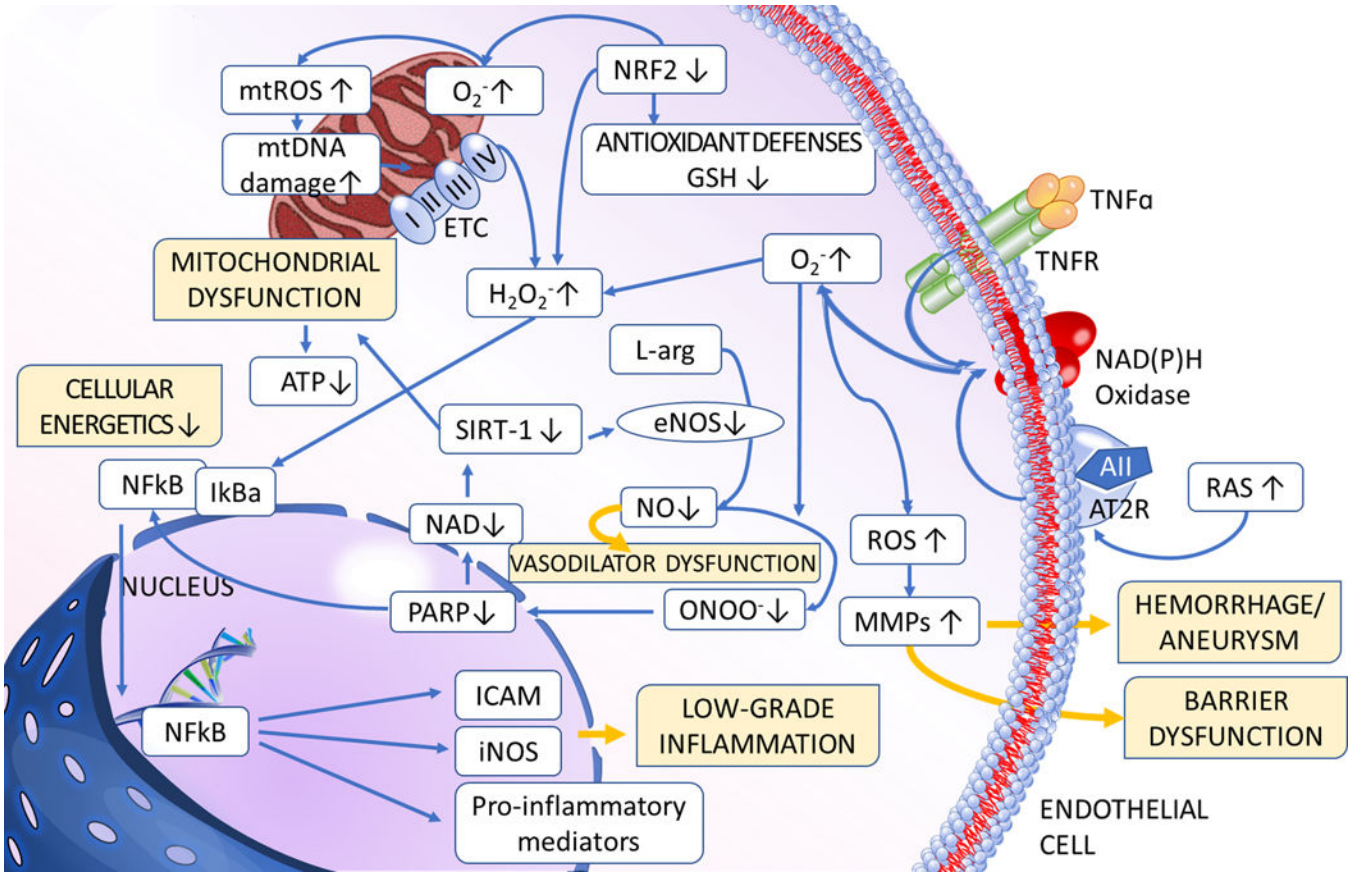

Figure 3: Mechanisms and consequences of age-related vascular inflammation.

The model predicts that multiple pathways converge on activation of inflammatory processes in the vascular tissue. During aging increased ROS production, exacerbated by Nrf2 dysfunction, enhances NF-κB activation, which promotes inflammatory cytokine and chemokine expression, microvascular endothelial activation, leukocyte adhesion and extravasation. Increased nitrative stress promotes PARP1 activation, which contributes to impaired activity of anti-inflammatory sirtuins. Sterile inflammation in the vascular wall is also exacerbated by increased secretion of inflammatory mediators from senescent cells and danger-associated molecular patterns (DAMPs), which activate innate immune system effectors, including toll-like receptors (TLRs) and the NLRP3 inflammasome complex. The aging vasculature in humans is also affected by the high prevalence of endothelium-trophic persistent cytomegalovirus (CMV) infection. Inflammatory processes contribute to a wide range of macro- and micro-vascular pathologies affecting older people.

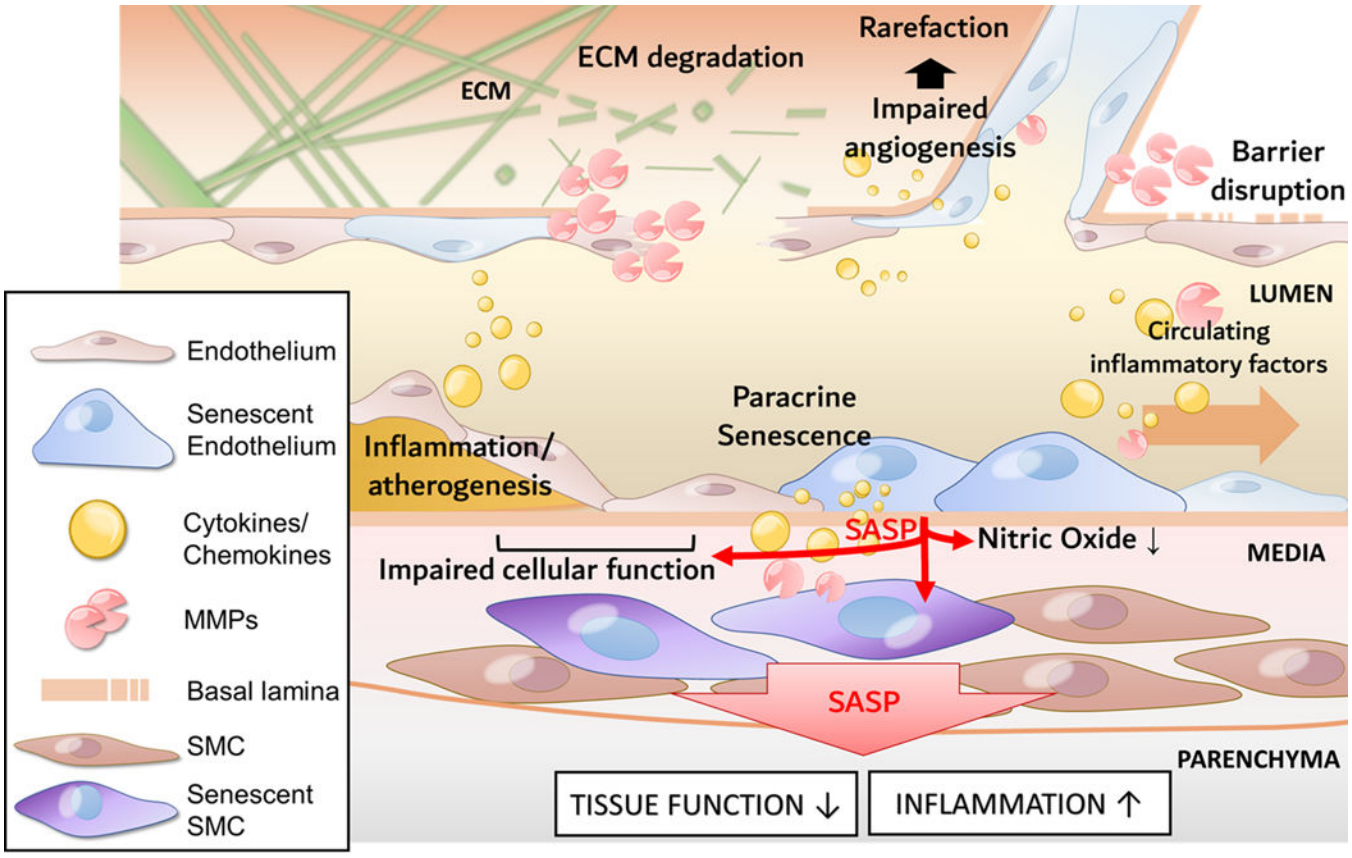

Figure 4: Conceptual model for the pathogenic role of cellular senescence in vascular aging.

The model predicts that increased presence of senescent endothelial and/or smooth muscle cell (SMC) in the aged vasculature and their proinflammatory secretome (SASP: senescence-associated secretory phenotype) contributes to impaired angiogenesis and microvascular rarefaction, pathological remodeling of the ECM, barrier disruption, chronic inflammation and atherogenesis.