Published in final edited form as:

Nat Rev Cardiol. 2023 January ; 20(1): 38–51. doi:10.1038/s41569-022-00739-0.

Mechanisms and consequences of endothelial cell senescence

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Abstract

Endothelial cells are located at the critical interface between circulating blood and semi-solid tissues and have many important roles in maintaining systemic physiological function. The vascular endothelium is particularly susceptible to pathogenic stimuli that activate tumour suppressor pathways leading to cellular senescence. In the past few years, the literature on cellular senescence has expanded and we now understand that senescent endothelial cells are highly active, secretory and pro-inflammatory, and exhibit an aberrant morphological phenotype. Moreover, endothelial senescence has been identified as an important contributor to various cardiovascular and metabolic diseases. In this Review, we discuss the consequences of endothelial cell exposure to damaging stimuli (haemodynamic forces, circulating and endothelial derived factors) and the cellular and molecular mechanisms that induce endothelial cell senescence. We also discuss how endothelial cell senescence causes arterial dysfunction and contributes to clinical cardiovascular diseases and metabolic disorders. Finally, we summarize the latest evidence on the impact of eliminating senescent endothelial cells (senolysis), and identify important remaining questions that can be addressed in future studies.

Introduction

The endothelium is a highly dynamic monolayer of cells that lines the vascular network. Endothelial cells initiate and dictate the formation of blood vessels via vasculogenesis. The

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Author contributions

S.I.B. and M.T.I. researched data for the article. All the authors contributed substantially to discussion of the content, wrote the article, and reviewed and edited the manuscript before submission.

Competing interests

A.J.D. is a scientific advisor and stockholder in Recursion Pharmaceuticals. None of the work done with Recursion is outlined or discussed in this Review. The other authors declare no competing interests.

Peer review information

Nature Reviews Cardiology thanks Jorge Erusalimsky and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

endothelium is also vital for maintaining blood in a fluid state and controls vascular tone through the production of vasoactive compounds, which is important for maintaining blood pressure and preventing orthostasis. Moreover, the endothelium is selectively permeable and mediates trafficking of macromolecules and immune cells out of the blood.

Endothelial cells are continuously exposed to many circulating factors and pathogenic stimuli that predispose them to damage, leading to impaired tissue and organ function via dysregulation of blood flow and barrier function. Furthermore, endothelial-cell-derived pro-inflammatory cytokines and chemokines transmit damaging signals to other vascular cells and non-vascular tissues and organs. A consequence of endothelial damage is cellular senescence, a state of permanent cell cycle arrest that occurs in response to a variety of stressors and acts to prevent uncontrolled proliferation of damaged cells and tumorigenesis.[1] However, senescence can also be deleterious, and a growing body of literature suggests that endothelial cell damage could lead to senescence and the development of a host of diseases.

Several lines of evidence provide the impetus for investigating endothelial cell senescence. Characteristics of vascular architecture, as well as the rate of endothelial turnover, are likely to predispose these cells to senescence. Tissues with a high proportion of endothelial cells have been identified as having the greatest burden of senescent cells,[2] and endothelial cells are known to be one of the first cell types to become senescent with advancing age.[3] In vivo, endothelial senescence occurs in multiple vascular beds including the kidney,[4] the retina,[5] the liver,[3] the brain[6] and the aorta,[7,8] suggesting that endothelial senescence contributes to a variety of pathological processes associated with vascular dysfunction. The development of models that allow for selective ablation of senescent cells, as well as drugs that selectively kill senescent cells (senolytics), has provided additional evidence that senescent endothelial cells represent a viable therapeutic target.[9] Endothelial cellular senescence has, therefore, become a topic of intense investigation.

Evidence demonstrates that endothelial senescence impairs endothelium-dependent dilation, angiogenesis and barrier function. Furthermore, numerous studies have identified a role for endothelial senescence in atherosclerosis, heart failure, pulmonary hypertension and metabolic dysfunction. Thus, understanding the role of endothelial senescence in these settings could lead to the development of novel therapeutics for cardiometabolic diseases.

In this Review, we provide a detailed discussion of advances in the field of endothelial cell senescence, including the phenotype of senescent endothelial cells and the molecular mechanisms that contribute to or protect from senescence. We also discuss the consequences of an increased abundance of senescent endothelial cells and their contribution to arterial dysfunction and cardiometabolic diseases. We also summarize the latest evidence on approaches to senolysis; although several senolytic drugs have entered clinical trials, these studies have been reviewed in detail elsewhere[9,10,14–17] and are beyond the scope of this article.

Phenotype of senescent endothelial cells

Two main tumor suppressor pathways are upregulated in response to a variety of senescence-inducing stressors. These pathways include the p53/p21 and pRB/p16 pathways.[1] p21 and cyclin-dependent kinase inhibitor 2A (CDKN2A; also known as p16) enforce the senescent state and, therefore, are commonly used as markers of senescence. p21 and p16 are differentially activated depending on the cell type and the stressor.[11] However, in some cell types, convergence is evident between p21-positive and p16-positive senescent cells, whereby damage leads to an early rise in p21 that decreases with time, followed by a later rise in p16.[12,13] Although the specific kinetics of p21 and p16 in vivo remain elusive, evidence from mouse models suggests that cells with high levels of p21 or p16 represent distinct populations.[14,15] The severity, duration and type of damage determines the molecular response and cell fate. For example, minor damage leads to p53 stabilization, allowing transcription of p21 until the damage is repaired, after which cell cycle re-entry is permitted.[16–18] However, delayed activation of p21, or more severe or sustained damage leads to senescence, which is often associated with elevated p16 expression.[16–18] In addition to senescence, p53 induces apoptosis, which occurs in response to even greater stress than is required for senescence.[16,17] In healthy individuals, both senescence and apoptosis lead to clearance of damaged cells.[17] However, in pathological states and advanced age, the balance between senescence and clearance is disrupted due to excessive damage or immune dysfunction (immunosenescence), leading to an accumulation of senescent cells.[17]

In addition to p21 and p16, senescent cells can also be identified by elevated activity of lysosomal senescence associated β-galactosidase.[1,19] Moreover, additional markers of senesce have been identified in the past 2 years (for example, lysosomal and proliferative features, expression of senescence-associated genes), and a consensus has been reached that whenever possible a combination of two or more markers should be used to identify senescent cells with high fidelity.[20,21]

Senescent cells are highly active and characterized by the senescence-associated secretory phenotype (SASP), which is heterogeneous and determined by the cell type and mechanisms of senescence.[5–8] Canonical SASP factors are a collection of inflammatory chemokines, cytokines, growth factors and, in some cases, reactive oxygen species (ROS) secreted by senescent cells.[5] In endothelial cells, senescence produces a distinct morphological and secretory phenotype — senescent endothelial cells are flat, enlarged and are refractory to phenotypic changes and alignment in response to laminar shear stress[22]. Mechanistically, senescent endothelial cells increase adhesion to basement membranes preventing the correct alignment to blood flow[22] (FIG. 2). The functional consequences of increased adhesion are that cells resist denudation and can persist for long periods of time. Additionally, the inability of these cells to align to blood flow might contribute to the reductions in NO and increased inflammatory signalling seen in senescent endothelial cells.[23]

Interestingly, the SASP of endothelial cells mirrors age-related changes that are important mediators of arterial dysfunction[11], but were not originally attributed to senescence. Evidence suggests that endothelial senescence results in elevated expression of inflammatory cytokines and ROS akin to the canonical SASP. Thus, an increase in the

abundance of senescent endothelial cells could cause inflammation and oxidative stress that impair endothelial function and stiffen large arteries through secretion of SASP factors. The source of ROS in senescent endothelial cells has not been fully elucidated. However, evidence suggests mitochondrial dysfunction as a potential source. For example, senescent cells have impaired mitochondrial respiration and increased mitochondrial superoxide production compared with non-senescent cells.[24] Conversely, targeting mitochondrial ROS reduces senescence induced by hydrogen peroxide.[25] The pro-inflammatory transcription factor nuclear factor NF-kappa-B (NF-κB) is associated with impaired endothelial function[11,26] and seems to be involved in the endothelial cell SASP. For example, chronic exposure to the inflammatory cytokine tumour necrosis factor (TNF) results in premature senescence that is characterized by elevated NF-κB activity and ROS production.[27] In this setting, inhibiting NF-κB or treating cells with antioxidants delays senescence and prevents induction of the SASP.[27] Senescent endothelial cells also produce numerous inflammatory chemokines and cytokines including IL-1β,[28] IL-6,[27,29] IL-8,[27,30] C-X-C motif chemokine 11[31] and plasminogen activator inhibitor 1 (PAI-1),[32,33] and demonstrate reduced expression of the anti-inflammatory cytokine IL-10,[34] as well as increased expression of a variety of cellular adhesion molecules.[27,35–38] Despite observations that the endothelial SASP is pro-inflammatory, several studies have demonstrated an opposite, anti-inflammatory effect of senescence in endothelial cells,[39–41] highlighting the need for more research on this phenomenon both in vivo and ex vivo.

Remodelling that reduces the elastic properties of arteries, resulting in stiffening, contributes to the development of cardiovascular diseases (CVD). Senescent endothelial cells upregulate pathways involved in arterial remodelling[42], such as the transforming growth factor-β (TGF-β)[33,36] and matrix metalloproteinase (MMP) pathways,[25] which could contribute to arterial stiffening. Indeed, in young mice, a genetic DNA repair defect in endothelial cells increases endothelial cell expression of cyclin-dependent kinase inhibitor 1A (CDKN1A; also known as p21) and aortic remodelling and stiffening, thereby phenocopying advanced age.[43]

Evidence from endothelial cells[44] and other cell types[45] suggests that secreted SASP factors can induce senescence in neighbouring cells. Even if endothelial SASP production is insufficient to drive senescence in neighbouring cells, such as vascular smooth muscle cells (VSMCs), nitric oxide (NO) is reduced in pathways associated with arterial remodelling, and elevated production of inflammatory SASP factors and ROS from senescent endothelial cells could have widespread deleterious effects on other cells in arteries and peripheral tissues.

Origins of endothelial senescence

Owing to their anatomical location, endothelial cells are continuously exposed to flowing blood containing many substances that can contribute to senescence, including oxygen, glucose, lipids, amino acid metabolites, immune cells, ROS and inflammatory cytokines[46] (FIG. 1). The haemodynamic forces of blood flow, such as shear stress and high or pulsatile pressure, can also induce senescence. Endothelial cell turnover is high in areas exposed to disturbed blood flow; therefore, these cells experience damaging haemodynamic and replicative stress. In regions that do not experience disturbed blood flow, endothelial cells

are long lived,[47] with some cells estimated to survive for up to 80% of the average human lifespan. [48] However, long-lived cells accumulate damage through prolonged exposure to harmful substances in the blood. Therefore, both short-lived and long-lived endothelial cells can become senescent through a variety of stimuli. The various environmental challenges that contribute to senescence are discussed in the following sections.

Haemodynamic environment

The location of endothelial cells within the vascular tree dictates the haemodynamic environment. Within large conduit arteries, endothelial cells are exposed to high blood pressure and pulsatile circumferential stresses, both of which can induce damage. Endothelial cells at the sites of branches and curvatures, such as the carotid sinus and aortic arch, experience low and oscillatory shear stress, which is deleterious and leads to a phenotype akin to the SASP[49,50]. By contrast, endothelial cells in the distal internal carotid artery or descending thoracic aorta are exposed to high laminar shear stress, which exerts beneficial effects.

The importance of the haemodynamic environment is highlighted by studies of cell cultures and mouse arteries, which show that endothelial cells exposed to disturbed flow display high cellular turnover, leading to replicative senescence.[50,51] Furthermore, evidence from human tissue samples shows that cell turnover and replicative senescence are greater in endothelial cells proximal to arterial bifurcations than in endothelial cells from arteries that experience less haemodynamic stress, venous endothelial cells and VSMCs.[52] The effects of exposure to damaging haemodynamic environments could explain the increased burden of atherosclerosis at sites of low and oscillatory shear stress. This notion is supported by the higher abundance of senescent endothelial cells in human atherosclerotic plaques compared with healthy vasculature.[38]

Oxygen and metabolites

At rest and normoxia, the partial pressure of oxygen in the blood is approximately 100 mmHg in arteries and 40 mmHg in veins.[53,54] By contrast, the interstitial partial pressure of oxygen is approximately 66 mmHg in the spleen[53], 34 mmHg in skeletal muscle[54] and 25 mmHg in the heart, liver and kidney[53]. The gradient between partial pressure of oxygen in arterial blood and peripheral tissue is greatly exaggerated during times of increased metabolic demand, such as exercise, when intracellular oxygen in peripheral tissues can be as low as 3 mmHg.[54] Cellular exposure to high partial pressure of oxygen is well known to hasten the onset of senescence.[55–59] Therefore, endothelial cells within the arterial tree continuously face a senescence-inducing stimulus not experienced by other cells within the body, which might explain their early onset of senescence. As such, it is important to consider that most cell culture experiments occur in supraphysiological oxygen conditions, and that mouse cells are more susceptible than human cells to oxidative DNA damage-induced senescence.[57,60]

Endothelial cells are continuously exposed to nutrients, metabolites and hormones (such as glucose, lipids, amino acids and insulin) and the concentration of these solutes increases postprandially.[61,62] Likewise, pathological states such as obesity and diabetes

mellitus, as well as advancing age, result in elevated circulating levels of insulin, glucose, triglycerides, cholesterol and amino acids[61–64], which could contribute to endothelial cell senescence.[8,65–72] Indeed, culturing endothelial cells with high levels of insulin results in premature senescence and reduced proliferation.[73] Mechanistically, insulin activates the RAC-alpha serine/threonine-protein kinase (AKT1, also known as Akt) signalling pathway that inhibits forkhead box protein O3a resulting in reduced manganese superoxide dismutase activity and a subsequent increase in ROS.[73] Several studies have demonstrated that glucose promotes endothelial cell senescence and SASP production both in vivo and in vitro.[8,65,68–70] Culturing endothelial cells with high levels of glucose suppresses the expression of sirtuins and histone acetylases, as well as the activity of forkhead box protein O1, resulting in oxidative stress.[69,70] Elevated ROS attenuates NO bioavailability, induces DNA damage and activates the cellular tumour antigen p53 (p53)–p21 signalling pathway, inducing proliferative arrest and cell senescence.[73] Evidence also exists that high glucose levels reduce telomerase activity, telomere length and phosphorylation of endothelial NO synthase (eNOS) thereby reducing NO production, all of which foster endothelial cell senescence.[8,66,74]

Amino acid metabolites and lipids have also been shown to induce endothelial senescence in culture[75–78] and in nonhuman primates.[79] Homocysteine, which is produced during metabolism of the amino acid methionine, hastens the onset of senescence.[78,80–82] In cultured human umbilical vein endothelial cells, chronic homocysteine treatment upregulates markers of senescence, due to demethylation of telomerase reverse transcriptase resulting in telomere shortening[78] (discussed later in this Review). In mice, a high methionine diet increases serum homocysteine levels and promotes several features of vascular aging, including arterial senescence.[83] Interestingly, reducing methionine is one of few interventions that has been shown to extend the lifespan of an organism in numerous models,[84] which could be partly explained by a delay in the onset of endothelial cell senescence. The interaction between circulating lipids and the endothelium is also vital to our understanding of how senescence promotes disease. Atherosclerosis is initiated by endothelial dysfunction, leading to deposition of lipids underneath the endothelial barrier.[85] Endothelial cell senescence could contribute to this lipid deposition, due to reduced expression of junctional proteins and impaired barrier function.[86,87] Together, these findings suggest that the elevated levels of insulin, glucose, amino acids and lipids caused by physiological dysfunction and pathological states lead to endothelial cell senescence that, in turn, contributes to disease progression.

Vasodilators and vasoconstrictors

Endothelial cells produce numerous vasodilators and vasoconstrictors, and have receptors for circulating vasoactive substances of non-endothelial origin. Notably, endothelial cells produce the potent vasodilatory molecule NO, which diffuses to VSMCs to induce vasodilation, reduces endothelial expression of adhesion molecules, improves barrier function, prevents coagulation and is generally vasoprotective. Furthermore, evidence suggests that NO could be the key vasodilatory molecule in the prevention of cell senescence.[67,74] Bradykinin is a vasoactive peptide that induces vasodilation via increased production of NO and other endothelial-derived relaxing factors. Importantly,

pharmacological inhibition of NO production impairs the senescence-blocking effects of bradykinin[88]. This finding is bolstered by evidence from cell culture studies that inhibition of eNOS increases senescence.[67,89] Importantly, NO production is impaired in senescent endothelial cells[90]; therefore, a lack of NO results in endothelial senescence that further reduces NO bioavailability — a hallmark of arterial aging that contributes to the progression of numerous diseases[74].

Other vasoconstrictors with senescence-inducing effects in endothelial cells include endothelin-1, which also impairs endothelium-dependent dilation (EDD)[91,92], and angiotensin-2[35,93–95]. The angiotensin-receptor blocker valsartan reduces angiotensin-2-induced senescence[35], indicating that the effects of this vasoconstrictor are mediated by type 1 angiotensin-2 receptors. Interestingly, repeated passaging of endothelial cells (a cell culture model of aging) increases their susceptibility to angiotensin-2-induced senescence.[35] Together, these findings suggest a feedforward mechanism by which vasodilators and vasoconstrictors modulate endothelial cell senescence, leading to changes in bioavailability of NO and physiological dysfunction.

Inflammation and oxidative stress

Inflammation and oxidative stress derived from many sources, including immune cells, endothelial cells and circulating factors produced by other tissues and exogenous sources, lead to endothelial dysfunction, which could be partly caused by senescence. In support of this concept, evidence suggests that exposure of endothelial cells to TNF induces premature replicative senescence[27,96] and increases endothelial cell expression of cytokines, chemokines[96,97] and adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion protein 1 (VCAM-1).[96] TNF-induced premature senescence and the consequential inflammatory milieu are suppressed by the administration of antioxidants or inhibition of NF-κB.[27] Likewise, treating endothelial cells with antioxidants in combination with co-factors necessary for enzymatic production of NO, partially prevents glucose-induced senescence.[67] Mechanistically, ROS and inflammatory factors are likely to drive endothelial senescence through DNA damage and activation of DNA damage-response pathways.[96] Senescent endothelial cells act as a source of inflammatory cytokines and ROS, which could induce senescence in healthy neighbouring cells in a feedforward fashion, thereby propagating and perpetuating the inflammatory microenvironment.[44] Collectively, this evidence suggests a complex interplay among inflammation, oxidative stress and endothelial cell senescence.

Iatrogenic senescence

Medical treatment that leads to cell senescence is known as iatrogenic senescence. Notably, endothelial dysfunction[98,99] and CVD[100] resulting from cancer therapy could, in part, be caused by endothelial cell senescence. This notion is supported by the fact that several chemotherapeutic drugs induce endothelial senescence in culture[29,31,32,101] and are administered to patients intravenously, thus interacting directly with endothelial cells (FIG. 1). As would be expected with induction of endothelial cell senescence, treating mice with the chemotherapeutic drug, doxorubicin, impairs EDD due to increased mitochondrial ROS[102] and leads to elevated expression of arterial SASP factors IL-1β and IL-6.[103]

Furthermore, genetic ablation of senescent cells following doxorubicin treatment improves EDD, suggesting a direct connection between chemotherapeutics and endothelial cell senescence.[104] Another chemotherapeutic drug — the vascular endothelial growth factor inhibitor, axitinib — has also been shown to induce endothelial cell senescence via ROS, in human umbilical vein endothelial cells.[101,105]

The clinical consequences of chemotherapy on the cardiovascular system often do not present until over a decade after treatment,[106] which supports the idea that accumulation of senescent endothelial cells contributes to CVD. Another consequence of chemotherapy-induced senescence in endothelial cells is the production of SASP factors that can promote tumour growth.[31] Thus, targeting endothelial cell senescence using senolytic drugs could be an effective strategy to prevent cancer progression acutely and reduce the risk of chemotherapy-related CVD. Senolytics are currently being evaluated for use in an ongoing clinical trial in survivors of childhood cancer.[107]Although the primary outcomes of this trial are unrelated to endothelial function or CVD development, if senolytics prove to be safe in this setting, future studies examining endothelial function prior to and after administration of senolytics, as well as long-term studies on the future incidence of CVD, would be of great interest and importance.[9]

Mechanisms of endothelial senescence

The damaging stimuli described above induce endothelial senescence through a variety of cellular and molecular mechanisms. These include telomere dysfunction, non-telomeric DNA damage, and alterations to energy-sensitive pathways. Consequently, cells activate tumour-suppressor pathways that permanently inhibit cell cycle progression, undergo morphological changes and upregulate production of SASP factors (FIG. 2).

Telomeres and DNA damage

In large arteries, endothelial cell turnover occurs primarily via mitotic cell division rather than replacement by progenitor cells.[108] A consequence of increased cell division without progenitor replacement is telomere attrition, which increases the likelihood of replicative senescence.[52] Telomeres at the ends of chromosomes are comprised of repeat sequences (TTAGGG) that shorten with each cell division due to the nature of the DNA replication machinery.[109] When telomeres become critically short, they lose the ability to maintain the telomere-loop (T-loop) structure that caps chromosome ends.[110] Telomere uncapping initiates a DNA damage response that activates the p53–p21 tumor suppressor pathway leading to cellular senescence.[110]

The contribution of telomere shortening to endothelial senescence is supported by evidence that disturbed blood flow shortens endothelial cell telomeres in culture.[50] A similar phenotype is seen in humans, whereby telomere attrition is greater in vascular regions exposed to disturbed flow than in regions that experience laminar flow.[52] These studies did not directly demonstrate that telomere attrition was sufficient to reach replicative senescence. However, as would be expected with critically short telomeres, disturbed flow increases endothelial cell senescence in mice and cell culture via the p53–p21 pathway[51], which is prevented by exogenous activation of telomerase (the enzyme that elongates

telomeres).[38] Attrition due to replication might only be partially responsible for critical shortening of telomeres at these sites, however, because telomere attrition is also accelerated by oxidative stress[111,112], which is elevated in regions of high endothelial cell turnover.[49]

In regions of laminar blood flow, endothelial cells are thought to be long lived and experience prolonged exposure to damaging agents. Telomeres are prone to oxidative DNA damage because their sequence contains an abundance of guanine triplets (TTAGGG), which are oxidation targets.[113,114] Telomere oxidation can lead to telomere uncapping and cellular senescence, independent of telomere length[115–117]. This concept is supported by the finding that older individuals and patients with hypertension have higher levels of telomere uncapping associated with p53–p21-induced senescence than younger and normotensive control individuals, irrespective of telomere length.[117,118] Although the precise mechanism leading to telomere uncapping remains unknown, evidence from mouse models supports the idea that uncapping due to replication and oxidative stress both lead to endothelial dysfunction. For example, genetic induction of telomere shortening or telomere uncapping results in an aged arterial phenotype in young mice.[119,120] The role of telomere dysfunction in vascular aging has been reviewed in more-depth elsewhere.[116,121,122] In addition to telomere dysfunction, non-telomeric DNA damage induces endothelial senescence both in vitro[37,123,124] and in vivo.[43] Akin to the consequences of telomere uncapping, studies have shown that impairments in the DNA damage repair response lead to an accelerated arterial aging phenotype in young mice.[43]

Sirtuins

Sirtuins (SIRTs) are NAD^+-dependent deacetylases and ADP-ribosyl transferases.[125] In response to elevated cellular NAD^+, SIRT1 and SIRT6 promote cellular repair and stress resistance by mediating tumour-suppressor and DNA damage-repair pathways.[125] SIRTs have, therefore, emerged as important mediators of endothelial cell senescence.

Exposure to oxidative or replicative stress reduces endothelial expression of both SIRT1 and SIRT6.[37,126–128] Additionally, inhibition of SIRT1 and SIRT6 increases endothelial cell senescence in culture,[37,127–129] suggesting the presence of a positive-feedback loop that predisposes cells to senescence over time through replication. Inhibition of SIRT1 in endothelial cells increases p53 activation,[129] which might explain how SIRT1 mediates endothelial cell senescence. Conversely, the mechanisms by which a reduction in SIRT6 leads to senescence seem to be upstream of p53–p21 activation, and are associated with the role of SIRT6 in telomere protection and DNA repair. For example, inhibition of SIRT6 in endothelial cells results in increased telomere uncapping[127,130] as well as in total nuclear DNA damage.[127] In concordance with the canonical role of SIRT6 as a mediator of heterochromatin and DNA, SIRT6 buffers against the induction of senescence by ROS,[126] which are known to induce DNA damage[123] and telomere dysfunction.[111] The totality of evidence suggests that SIRT1 and SIRT6 prevent endothelial senescence by protecting DNA and reducing activation of the p53–p21 senescence pathway.

SIRT3 also has a role in preventing endothelial cell senescence. This molecule is localized to the mitochondria and protects cells from oxidative stress.[125] Elevated SIRT3 expression reduces endothelial cell senescence in culture.[83,131] Conversely, levels of SIRT3 are reduced

in senescent endothelial cells.[131] Whole-body overexpression of SIRT3 protects mice from endothelial dysfunction induced by angiotensin-2 and reduces aortic senescence.[132] These findings allude to an important role for SIRT3 and mitochondrial function in endothelial cell senescence. Mitochondrial dysfunction has been implicated as an inducer and a consequence of senescence in many cell types.[133,134] However, the direct contribution of mitochondrial function to senescence within endothelial cells remains understudied. Endothelial cells are highly glycolytic, and do not rely heavily on oxidative phosphorylation for ATP production.[135] Nonetheless, alterations in mitochondrial function seem to precede the induction of senescence, as replicative aging of human brain microvascular endothelial increases reliance on oxidative phosphorylation for energy production.[136] Furthermore, in endothelial cells, radiation-induced senescence has been shown to increase mitochondrial ROS production, whereas protecting cells with antioxidants reduces the burden of senescence[24] by maintaining telomere length and reducing both lipid peroxidation and total nuclear DNA damage,[137] thereby extending replicative capacity.[138] Together, these findings suggest that molecular mediators of mitochondrial function, such as SIRT3, have a critical role in preventing endothelial senescence.

Consequences of endothelial senescence

The role of endothelial cell senescence on vascular function and in cardiometabolic diseases is summarized below and in FIG. 3.

Endothelium-dependent dilation

EDD is impaired with advancing age,[134] and is associated with CVD, morbidity and mortality.[134] Elevated oxidative stress and inflammation are implicated as causal factors in diminished EDD.[139,140] An increase in resident senescent cells could plausibly represent the origin of such oxidative stress and inflammation. Furthermore, senescent endothelial cells have reduced eNOS expression, which contributes to impaired EDD.[72,90,141,142] Although, no studies have yet demonstrated unequivocally that endothelial cell senescence alone is sufficient to disrupt EDD, many reports allude to this being the case. For example, in aged mice, treatment with the senolytic drugs dasatinib and quercetin improves carotid artery EDD and reduces the number of uncapped telomeres in aortic endothelial cells, with no change in the smooth muscle vasodilatory response to NO.[143] This study provides compelling evidence that reducing the burden of senescent cells in advanced age is particularly beneficial for endothelial function with little effect on smooth muscle. These data are supported by a study of vascular endothelial cells from healthy, sedentary humans, in which increased senescence was associated with impaired EDD.[144]

Telomere uncapping has also been associated with EDD. In mice, whole-body induction of telomere uncapping induces arterial senescence and reduces EDD and NO bioavailability due to elevated ROS production, despite having no effects on smooth muscle vasodilation in the presence of NO.[119] Furthermore, mice bred to have critically short telomeres accumulate senescent endothelial cells, exhibit oxidative damage in large arteries and reduced EDD due to oxidative stress, with a preserved smooth muscle vasodilatory response to NO.[120] In humans, arterial telomere uncapping is associated with senescence and expression of

inflammatory cytokines known to impair EDD.[117] These studies support the hypothesis that the endothelium is particularly susceptible to senescence-inducing stimuli, resulting in impaired EDD. However, the effect of senescence in other cell types that interact with the endothelium, or changes in circulating factors, cannot be discounted. Therefore, investigations that directly induce or prevent senescence selectively in the endothelium are warranted.

Angiogenesis

The process of forming new blood vessels, known as angiogenesis, is an essential physiological process. For example, adaptation to exercise requires angiogenesis to meet the elevated demand for oxygen and nutrients; therefore, impaired angiogenesis contributes to exercise intolerance seen in elderly adults.[145] Likewise, repairing cardiac or other tissues after injury requires angiogenesis, and disruption to this process delays and negatively impacts recovery.[145] Importantly, aging results in reduced angiogenic capacity of the visceral adipose tissue vasculature.[146] Endothelial cells are the main cell type that initiates and sustains the angiogenic process, primarily via vascular endothelial growth factor, fibroblast growth factor and hypoxia-inducible factor 1-α. Therefore, understanding age-related changes in endothelial cells that affect these signalling pathways and impair angiogenesis could reveal novel therapeutic targets.

Cellular senescence could be a fundamental mechanism underlying age-associated and disease-associated changes in angiogenesis. Experimental evidence demonstrates that disruption of telomeric repeat-binding factor 2 (TRF2) in endothelial cell senescence impairs angiogenic capacity.[147] Likewise, genetic deletion or inhibition of telomerase impairs angiogenesis in rodents, compromising tissue repair in a model of ischaemia–reperfusion injury.[148,149] Evidence also exists that ionizing radiation induces senescence and reduces angiogenic capacity in cerebrovascular endothelial cells.[150] Furthermore, genetic deletion of the gene for endothelial p53 (*Trp53)* ameliorates vascular density in a murine model of heart failure, implicating a role of endothelial senescence and angiogenesis in cardiac injury.[151] Mechanistically, endothelial cell senescence lowers *VEGF* gene expression, which could contribute to attenuated angiogenic capacity.[152]

Several lines of evidence support targeting cellular senescence to restore and maintain angiogenesis. For example, deletion of the genes for p21 (*Cdkn1a*)[153] or p53 (*Trp53*)[154,155] in mice attenuates endothelial cell senescence, and improves proliferation and angiogenesis.[156] Deletion of endothelial *Trp53* also results in improvement in angiogenesis, prevention of pressure overload-induced cardiac fibrosis and diabetic cardiomyopathy in mice.[154,155] Although these studies provide convincing evidence that endothelial senescence inhibits angiogenesis, a report published in 2021 paradoxically demonstrated that, in a mouse model of retinopathy, endothelial senescence induces pathological angiogenesis and expression of SASP factors that promote abnormal neovascularization and fibrosis[157]. Treatment with senolytics attenuates disease severity by reducing angiogenesis and allowing regrowth of functional blood vessels[157]. Taken together, the literature supports the assertion that endothelial cell senescence strongly influences angiogenesis and can be targeted therapeutically; however, future studies on this topic are warranted.

Barrier function

As the inner lining of blood vessels, the endothelium forms a selectively permeable barrier that moderates the passage of molecules between blood and peripheral cells and tissues.[158] Permeability of the endothelium is tissue specific and is mediated by junctional proteins.[158] Maintenance of barrier function is critical, as barrier breakdown is involved in numerous diseases.[158] Interestingly, culturing endothelial cells until they become senescent leads to disorganization of the junctional proteins cadherin-5 (also known as vascular endothelial cadherin) and tight junction protein ZO-1 and reductions in junctional proteins occludin and claudin-5.[87] Furthermore, a bystander effect is apparent, whereby non-senescent cells display dysfunctional tight junctions when co-cultured with senescent endothelial cells,[87] which could be explained by the SASP, as inflammation is known to increase permeability of the endothelial monolayer.[158] Importantly, induction of endothelial senescence impairs blood brain barrier function in vitro[86,87,159] and in vivo[86]. The consequences of endothelial cell senescence, and the subsequent impairments in barrier function, are exemplified in the disease states discussed below.

Hypertension

Hypertension is one of the greatest contributors to morbidity and mortality worldwide, and is associated with elevated oxidative stress and inflammation[160]. Endothelial dysfunction is implicated in the connection between oxidative stress, inflammation and the development of hypertension.[160] These processes negatively impact the ratio between vasoprotective dilatory molecules, such as NO, and vasoconstrictor molecules, such as angiotensin-2 and endothelin-1, thus contributing to increases in systemic vascular resistance and systolic blood pressure. Therefore, the molecular mechanisms and subsequent phenotype that characterizes senescent endothelial cells strongly indicate that endothelial cell senescence contributes to the development of hypertension. Although evidence of this link exists[161–163], few studies have demonstrated a direct cause-and-effect relationship between endothelial cell senescence and hypertension.

Perhaps the most convincing area of research pointing to senescence as a driver of hypertension is the study of telomere dysfunction. For example, mice that are genetically bred to have critically short telomeres are hypertensive due to elevated endothelin-1 levels.[164] Moreover, genetic induction of telomere uncapping, independent of telomere length, results in arterial senescence and elevated systolic blood pressure in mice.[119] Human patients with hypertension were found to have significant increases in telomere uncapping, which was associated with cellular senescence, when compared with normotensive individuals.[118] This finding is supported by evidence that, of all the risk factors for atherosclerosis, hypertension is the best predictor of endothelial senescence.[161]

As well as having a causative role in hypertension, endothelial senescence could also be a consequence of increased blood pressure because pro-hypertensive stimuli, including endothelin-1 and angiotensin-2, induce endothelial cell senescence.[66,35,93–95] Moreover, elevated pulse pressure exposes endothelial cells to mechanical stress that can drive senescence, as demonstrated by murine studies of transverse aortic constriction, which results in acute increases in pulse pressure leading to p53–p21-mediated senescence in

the brain microvasculature.[165] These data suggest a positive feedback loop, whereby senescence reduces NO and increases endothelin-1 and angiotensin-2, which induces more senescence that further elevates blood pressure and leads to even greater senescence. The role of endothelial cell senescence as a stand-alone mechanism in hypertension requires further investigation. To this end, mouse models of endothelial cell-specific induction of senescence, such as telomere uncapping, or manipulation of p16, p21 or p53, could be instrumental. Likewise, genetic or pharmacological ablation of senescent endothelial cells in models of hypertension (for example, high-salt high-fat diet, or eNOS inhibition) could shed light on the basic and translational value of targeting endothelial cell senescence in hypertension.

Atherosclerosis

Atherosclerosis is an inflammatory disease initially characterized by development of a fatty streak in the lumen of arteries that progresses to the development of advanced plaques containing oxidized lipids, a necrotic core and neovessels.[85,166] The severity of disease is determined by the size of the plaque and its composition, which determines stability.[167] Unstable plaques with thin fibrous caps are prone to rupture and, in the face of altered haemodynamic forces, become dislodged and occlude capillaries resulting in clinical cardiovascular events, such as myocardial ischaemia and infarction, stroke and peripheral artery diseases.[85,167] Endothelial cells have a critical role in the formation and progression of atherosclerotic plaque, and in plaque stability and rupture.[85] A healthy endothelial monolayer forms tight junctions preventing blood-borne factors, such as LDL-cholesterol (LDL-C) and immune cells, from entering the subintimal space.[166,167] Likewise, endothelial cells prevent immune cell adhesion and rolling as well as platelet aggregation via secretion of NO.[167] However, in atheroprone areas of the arterial tree, endothelial dysfunction is the first detectable change that occurs in response to damaging stimuli.[85] Endothelial dysfunction attenuates NO production and impairs barrier function, fostering LDL-C and immune cell infiltration.[167] Moreover, activation of endothelial inflammatory signals and ROS promotes disease progression and contributes to plaque instability.[167] Endothelial cells within atherosclerotic plaques form leaky blood vessels, known as vasa vasorum, facilitating nutrient delivery, immune cell infiltration and haemorrhage, which accentuate plaque growth and instability.[167]

Many features of endothelial cell dysfunction in atherosclerosis are akin to the senescent phenotype. The first evidence on the role of endothelial cell senescence in atherosclerosis came from an observational study published in 2002, which demonstrated that endothelial senescence is upregulated in human atherosclerotic plaques.[38] Over the past 20 years, more studies have been performed elucidating the causal role, mechanisms and the therapeutic potential of endothelial cell senescence in atherosclerosis. For example, together with macrophage foam cells and VSMCs, endothelial cells have been identified as vital contributors to atherosclerotic plaque formation and stability.[168] In support of a causal role of endothelial senescence in atherosclerosis, induction of endothelial cell-specific senescence by overexpressing a dominant negative TRF2 promotes atherosclerosis, without affecting plasma triglyceride levels.[169] Mechanistically, senescent endothelial cells contribute to atherosclerotic disease progression by impairing barrier function,[87] resulting in

infiltration of LDL-C and immune cells.[87,170] Likewise, endothelial senescence attenuates NO production and increases NADPH oxidase and prostaglandin G/H synthase 2 (also known as cyclooxygenase-2) production, leading to platelet activation, adhesion and aggregation.[85,171,172] Attenuated NO production promotes leukocyte rolling, adhesion and infiltration[90,170], increases VSMC contractility and proliferation[85,173] and reduces red blood cell oxygen delivery,[85] all of which foster atherosclerotic diseases progression.

As discussed earlier, senescent endothelial cells secrete a host of SASP factors including inflammatory cytokines, matrix proteins and proteases and growth factors, all of which are known to contribute to atherosclerosis. Notably, compared with healthy cells, senescent endothelial cells produce higher levels of VCAM-1 in response to TNF and lipopolysaccharide in an NF-κB-dependent manner.[169] Endothelial cells from human and murine atherosclerotic plaques also upregulate neurogenic locus notch homolog protein 1 signalling, which promotes ICAM-1, IL-6, IL-8 and IL-1α expression and transendothelial migration of monocytes.[174] In addition to promoting plaque growth, compelling evidence suggests that senescent cells also drive plaque instability via the production of MMPs.[168] However, these findings are from a study of MMP production in a mixed population of senescent cells from atherosclerotic plaques. Therefore, the individual role of endothelial cell senescence in plaque instability requires future investigation. Assuming that inducing apoptosis in senescent endothelial cells does not adversely affect plaque stability, clearing senescent endothelial cells is a potential therapeutic target for treating atherosclerosis. Studies have shown that elimination or inhibition of senescent endothelial cells using genetic ablation[180] or senolytics[76,168]or via inhibition of angiopoietin-related protein 2 (also known as angiopoietin-like 2)[175] and cyclin-dependent kinase 5[176], or activation of peroxisome proliferator-activated receptor-α[177] can suppress atherosclerotic disease progression. Collectively, these studies suggest that endothelial cell senescence not only contributes to, but also represents a viable therapeutic target for atherosclerosis

Heart failure

Heart failure is characterized by reductions in cardiac muscle function that impairs filling of the heart chambers (diastolic dysfunction, known as heart failure with preserved ejection fraction: HFpEF) or the ability to properly eject blood (systolic dysfunction, known as heart failure with reduced ejection fraction: HFrEF).[178]

In HFpEF, endothelial dysfunction akin to the senescent phenotype has been proposed to contribute to disease progression.[179] Comorbidities, such as diabetes, and risk factors, such as advanced age (both of which induce endothelial senescence), promote endothelial-derived inflammation and oxidative stress within the coronary circulation, which impairs EDD due to reductions in NO and contributes to pathological remodelling.[179] Although direct evidence is sparse, several studies support a role for endothelial senescence in HFpEF. For example, in a mouse model of accelerated senescence, a high-fat high-salt western diet exacerbated endothelial senescence, inflammation and impaired NO-mediated vasodilation, ultimately resulting cardiac hypertrophy, fibrosis and reduced diastolic function.[180] Conversely, systemic inhibition of p53 in a mouse model of diabetes ameliorates cardiac inflammation, oxidative stress and subsequent remodelling[155], which

could result partly from the prevention of endothelial cell senescence.[151,154] Additional evidence that endothelial senescence is a potential therapeutic target in HFpEF comes from studies of aged mice, in which genetic or pharmacological clearance of senescent cells reduces cardiac hypertrophy and fibrosis.[115] Although both methods of senescent cell ablation in this study targeted multiple cell types, the senolytic (navitoclax) is known to selectively kill senescent, but not non-senescent endothelial cells.[181] Effective pharmacotherapies for HFpEF are lacking. Targeting cells other than the cardiomyocytes could be a promising, novel approach, as HFpEF is the result of dysfunction within multiple organ systems, including the vasculature.[182] Furthermore, concerns about a potential lack of cell replacement after clearance of senescent cardiomyocytes (although post-mitotic, cardiomyocytes display many features of senescence)[183] means that senolytics targeting endothelial cells could be advantageous.

Evidence in the literature also supports the role of endothelial senescence in HFrEF. For example, in a transverse aortic constriction mouse model of cardiac hypertrophy and HFrEF,[184] the p53–p21 pathway is upregulated in endothelial cells.[151] Conversely, blunting the induction of senescence via genetic deletion of *Trp53* specifically from endothelial cells reduces expression of the SASP factors PAI-1, TNF, C-C motif chemokine 2 (also known as monocyte chemotactic protein 1) and the adhesion molecule ICAM-1, thereby decreasing cardiac fibrosis and cardiomyocyte hypertrophy, resulting in improved systolic function and survival.[151,154] These findings are supported by a mouse model of ischaemia–reperfusion injury that impairs systolic function and recapitulates the increased risk of heart failure after myocardial infarction.[185] Treatment with the senolytic navitoclax attenuated impaired systolic function and infarct size by increasing proliferative capacity of endothelial cells and, thus, stimulating angiogenesis.[185] Collectively, these studies indicate that endothelial cell senescence contributes to both HFpEF and HFrEF and senolytics that ablate senescent endothelial cells are beneficial for cardiac function. Despite these encouraging findings, the use of senolytics in this setting could affect other cell types that lack regenerative potential[183,186] and so additional research in this area is warranted.

Pulmonary hypertension

Pulmonary hypertension has a broad range of aetiologies, from rare and idiopathic, to common and occurring as a complication of cardiovascular or respiratory conditions.[187] Numerous cell types are involved in the pathophysiology of pulmonary hypertension. However, studies have identified endothelial cell senescence as an important contributor to the development of this disease.[187] Senescent endothelial cells have been identified in rodent models of pulmonary hypertension, cell cultures from patients with pulmonary hypertension and single-cell RNA sequencing data from lung tissue,.[188,189] Increased susceptibility to damage is a likely cause of endothelial cell senescence in pulmonary hypertension. For example, cultured lung endothelial cells from patients with pulmonary hypertension are more susceptible to disturbed blood flow and radiation-induced senescence compared with controls.[188] Another study has provided further mechanistic insight into endothelial cell senescence in pulmonary hypertension. The researchers found that deficiency in the mitochondrial iron–sulfur cluster biogenesis protein, frataxin, leads to endothelial cell

senescence, which increases inflammation and vascular remodelling and contributes to pulmonary hypertension.[189]

In pulmonary hypertension, endothelial cell senescence seems to drive dysfunctional phenotypes through upregulation of several canonical SASP factors, such as IL-6, TNF and PAI-1, leading to dysfunction in other vascular cells.[188,189] For example, conditioned media derived from the senescent endothelial cells of patients with pulmonary hypertension increased inflammatory markers and proliferation in non-senescent smooth muscle cells.[188,189] Importantly, the consequences of endothelial senescence in pulmonary hypertension are attenuated by treatment with senolytics, which reduce the burden of senescence and expression of SASP factors and improve clinical manifestations of pulmonary hypertension, such as reducing medial hypertrophy and pulmonary blood pressure.[188,189] Further studies are warranted to examine the impact of eliminating senescent cells in pre-clinical models of pulmonary hypertension and in clinical trials of patients with this condition.

Metabolic function

Systemic metabolic disorders, such as hyperglycaemia and dyslipidaemia, drive vascular dysfunction.[190] However, emerging evidence demonstrates that endothelial cell senescence and dysfunction precedes and, in many instances, has a causal role in these conditions. For example, endothelial cell-specific inactivation of NF-κB prevents metabolic dysfunction induced by a high fat diet by suppressing vascular senescence and the SASP.[191] Evidence also exists that endothelial upregulation of p53 and induction of senescence attenuates phosphorylation of AKT1, indicating an impairment in insulin signalling.[72] Another study revealed that conditioned media from senescent endothelial cells increases SASP factors and oxidative stress in adipocytes.[44] Furthermore, this treatment attenuated protein expression of insulin receptor substrate 1, leading to reduced insulin stimulated phosphorylation of AKT1 in adipocytes, consistent with impaired insulin signaling.[44] In mice, endothelial cell-specific *Trp53* deletion reduces obesity, systemic glucose intolerance and insulin resistance induced by a high-fat diet[72]. Conversely, endothelial cell-specific upregulation of p53 exacerbated these adverse metabolic phenotypes, demonstrating the critical role of endothelial senescence in systemic metabolic function.[72] In another study, endothelial cell-specific inhibition of the telomere capping protein TRF2 resulted in systemic insulin resistance, partly via circulating SASP factors.[44] Taken together, these findings indicate that endothelial cell senescence leads to inflammatory cytokine secretion that adversely effects neighbouring cells in a paracrine and endocrine manner, drives systemic metabolic dysfunction and can be targeted to improve metabolic function. The mechanistic link between endothelial cell senescence and systemic metabolic impairments is likely to be due to alterations in immune cell infiltration, blood flow, vasodilation, capillary density and capillary recruitment in metabolically active tissues such as adipose tissues and skeletal muscle. Further research into these processes is warranted.

Adverse effects of senolysis

Although eliminating senescent cells is generally thought to be beneficial, killing certain senescent cells with senolytics can be detrimental[192,183]. The deleterious effects of senolysis stem from systemic drug administration leading to the unintentional death of post-mitotic cells that express senescence markers, but that cannot be replaced. Evidence from a mouse model of heart failure suggests that clearance of senescent cells, using genetic (p16-positive cells) or pharmacological (navitoclax) approaches, attenuates hypertrophy of cardiomyocytes and fibrosis[115,183]. However, these interventions also result in multinucleated cardiomyocytes, a potential cause of future cardiomyocyte hypertrophy and maladaptive remodelling.[115,183] The method of killing senescent cells seems to be an important determinant of adverse effects, as genetic and pharmacologic senolysis have been shown to have differential effects on atherosclerosis.[193] In this study, navitoclax, suppressed atherosclerosis and attenuated inflammation, but had no effect on senescence markers in vivo, suggesting a potential limitation of commonly used markers.[193] The systemic consequences of attenuating senescence are supported by evidence from mice, whereby whole-body deletion of p53 (*Trp53*$^{-/-}$) or p16 (*Cdkn2a*$^{-/-}$) exacerbates cardiac fibrosis in models of heart failure induced by transverse aortic constriction or left carotid artery ligation.[194,195] Moreover, genetic removal of p16-positive cells induces hepatic and perivascular fibrosis, as well as blood vessel permeability.[3] Collectively, the evidence suggests that effective elimination of senescent cells in a clinical setting requires the target cell type to be determined, and senolytics selective to that particular cell type and disease state to be used. Interestingly, quercetin and fisetin, two of the senolytic drugs currently being evaluated for use in clinical trials, were first identified based on their ability to selectively kill senescent, but not non-senescent, endothelial cells.[196,197]

Conclusions

Cellular senescence is a critical biological phenomenon whereby cells undergo permanent proliferative arrest, remain metabolically active, and become secretory in nature. Senescent cells have important roles in development and wound healing and are, therefore, essential for maintaining physiological homeostasis. In normal physiological conditions, senescent cells are cleared by immune cells preventing their accumulation over time. However, with advancing age and in pathological states, senescent cells accumulate in tissues and organs where they contribute to disease progression and physiological dysfunction. The development of genetically modified mouse models, which enable researchers to track and ablate senescent cells, as well as induce senescence, has greatly advanced our understanding of the role of cellular senescence in disease. Furthermore, the development of senolytic drugs demonstrated that cellular senescence is a viable therapeutic target, leading to numerous ongoing clinical trials.

In this Review, we have explored the idea that endothelial cells are particularly susceptible to cellular senescence due to their anatomical location and exposure to unique mechanical and biochemical stimuli. We have also described the cellular and molecular mechanisms and consequences of endothelial cell senescence, as well as the physiological effects in

the vasculature and in diseases that encompass multiple tissue types and organ systems — specifically in cardiometabolic diseases.

General limitations of studies in the field leave some questions still to be addressed in future research. For example, many models are not cell-type specific — owing to interactions between endothelial cells, circulating factors and other cell types, studies using whole-body manipulations in mice, or clinical samples from humans, cannot rule out affects from other cell types. Although technically challenging, endothelial cell-specific manipulation will be required to improve our understanding of endothelial senescence in aging and disease. Investigations into how, and in what setting, senescence-inducing damage occurs would provide foundational knowledge about how endothelial cells become senescent. Genetically modified mouse models and technologies, such as lineage tracing, live-cell imaging, single-cell RNA-sequencing and other omics approaches, will be instrumental in furthering our understanding of how senescence is induced. This information is important because the method of senescence induction effects the SASP, which is largely responsible for the deleterious effects of increased senescence burden. Furthermore, understanding how senescence occurs could provide novel therapeutic opportunities to prevent senescence-inducing damage or kill senescent endothelial cells. From a translational perspective, ongoing clinical trials of senolytics will shed light on the role of endothelial cell senescence in human aging and disease.

Acknowledgements

The authors are supported by funding from National Institute of Health Awards R01 AG060395 (A.J.D.), R01 AG050238 (A.J.D.), R01 AG048366 (L.A.L.), F31AG076312 (S.I.B.) and Veteran’s Affairs Merit Review Award I01 BX004492 (L.A.L.) from the United States Department of Veterans Affairs Biomedical Laboratory Research and Development Service. The contents do not represent the views of the United States Department of Veterans Affairs, the National Institutes of Health or the United States Government.

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Key points

- Forming the inner lining of blood vessels, endothelial cells are exposed to a unique milleu of damaging stimuli, including haemodynamic forces as well as circulating and endothelial-derived factors.
- Exposure to damaging stimuli results in telomeric and non-telomeric DNA-damage, mitochondrial dysfunction and alterarations in energy sensor pathways in endothelial cells.
- Changes induced by damaging stimuli lead to the activation of tumour suppressor pathways, such as p53–p21 and pRb–p16, resulting in proliferative arrest and senescence.
- Senescent endothalial cells are enlarged, flat and refractory to changes in response to shear stress; they are also metabolically active and secrete a host of inflammatory molecules.
- Senescent endothelial cells and their secreted factors are major contributors to arterial dysfunction and the pathophysiology of various cardiometabolic diseases.
- Emerging evidence suggests that targeting senescent endothalial cells can be an effective strategy to supresses cardiometabolic diseases.

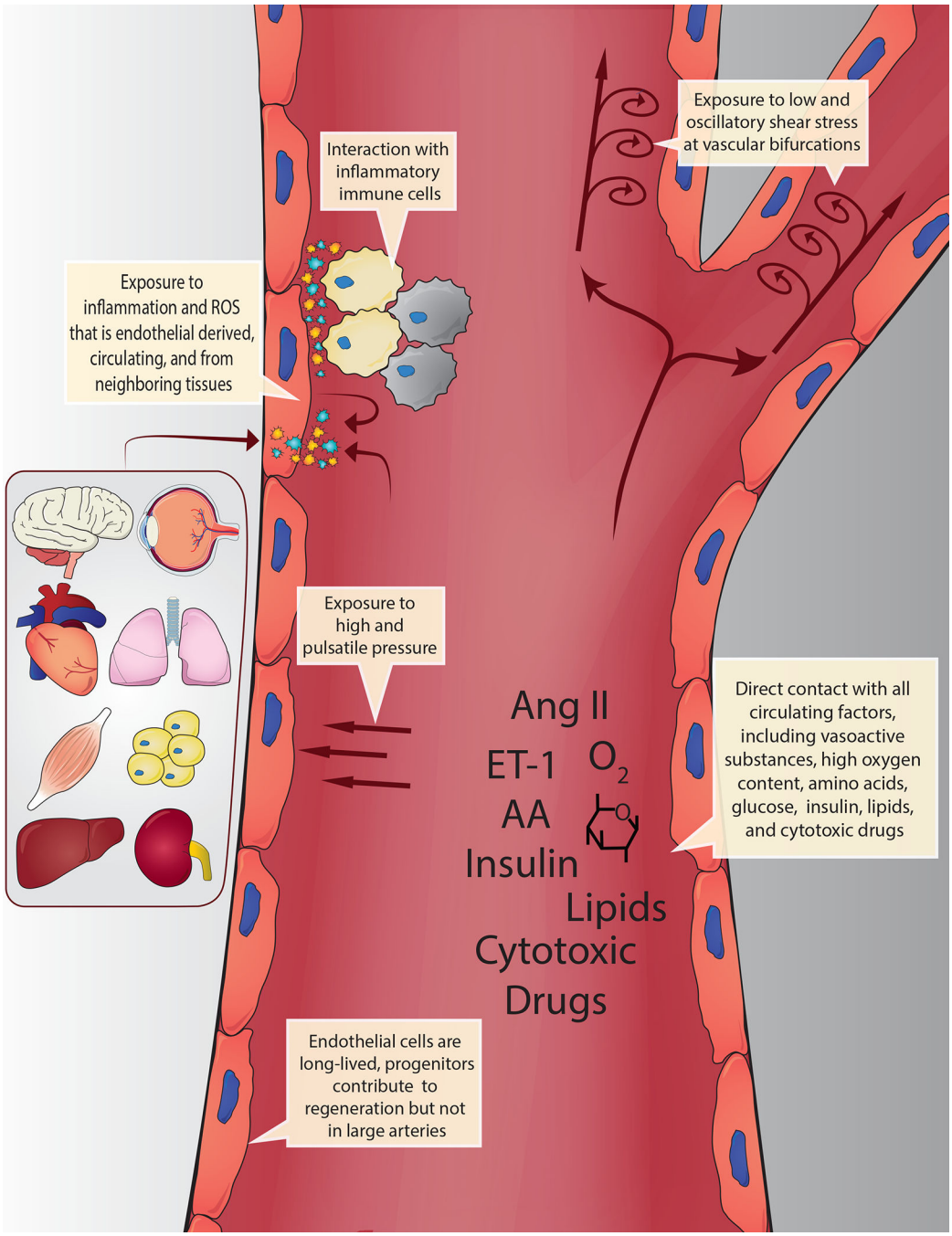

Fig. 1 |. Morphological changes in senescent endothelial cells.

Senescent cells (shown in blue) become enlarged and adhesion to basement membranes is increased, thereby hindering their alignment to laminar shear stress.

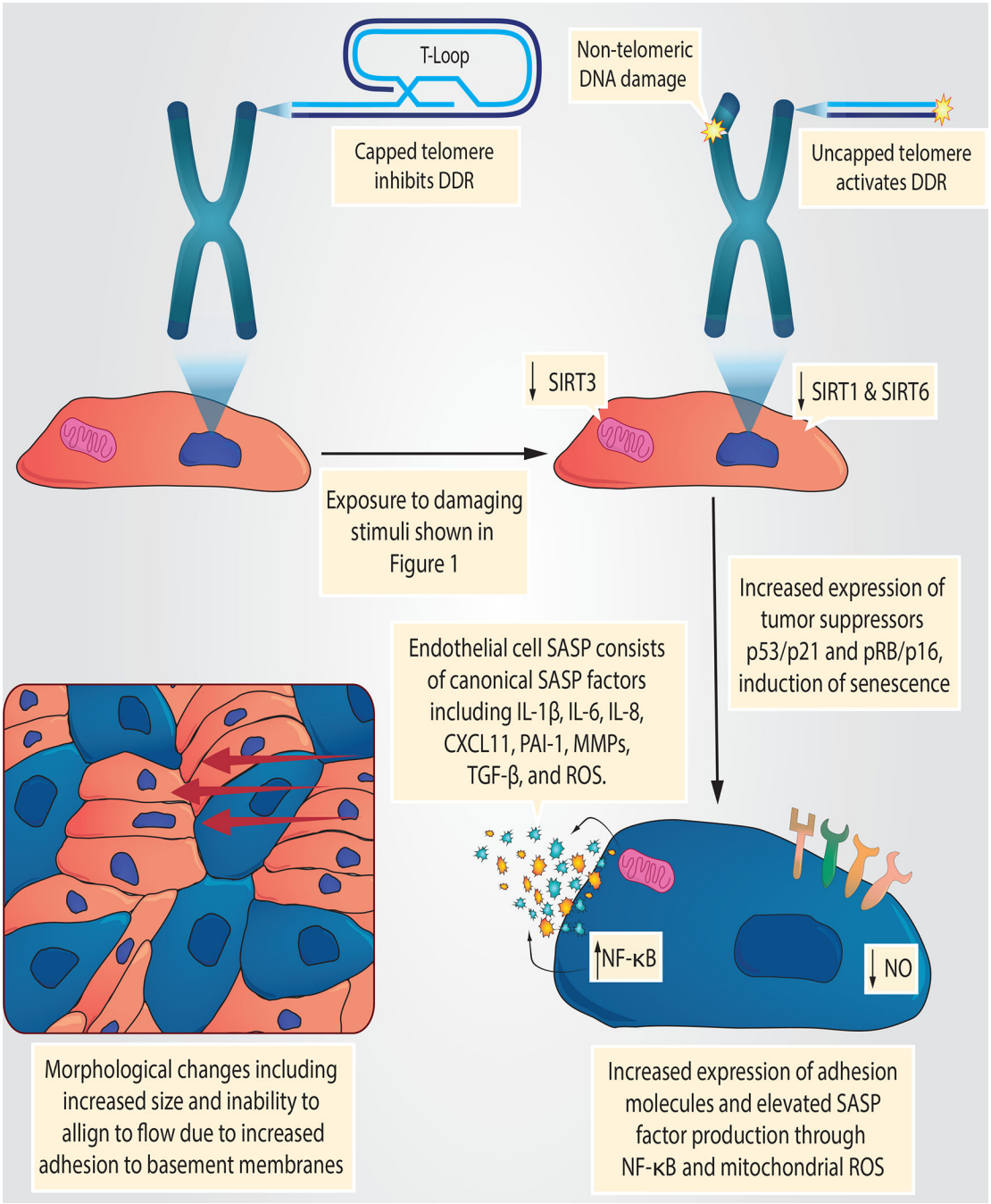

Fig. 2 |. Exposure of endothelial cells to circulating and endogenous stimuli.

Owing to their anatomical position, endothelial cells are continually exposed to circulating biochemical factors and haemodynamic forces. These factors include oxygen, nutrients (e.g. amino acids, glucose, hormones (e.g. insulin, angiotensin II, endothelin 1 and lipids) and cytotoxic drugs (e.g. chemotherapeutics). Haemodynamic forces include low and oscillatory shear stress at bifurcations and curvature points, as well as high and pulsatile pressure in other regions. Endothelial cells are also exposed to inflammatory cytokines and reactive oxygen species (ROS) derived from circulating immune cells and directly from every

organ and tissue. This combination of unique damaging stimuli renders endothelial cells particularly susceptible to cellular senescence. Moreover, these interactions are prolonged due to the long-lived nature of endothelial cells, particularly in large arteries where they are rarely replaced by progenitors.

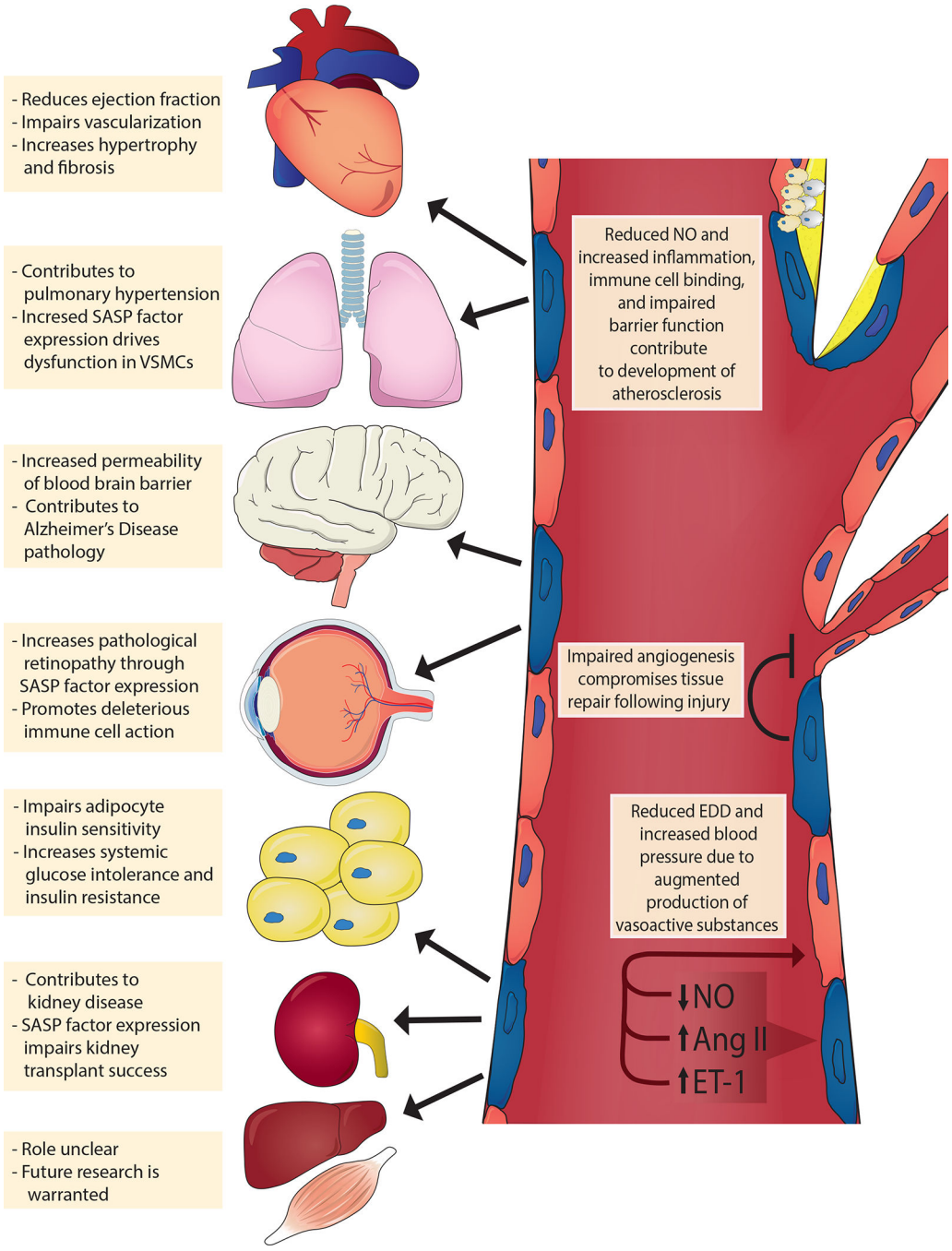

Fig. 3 |. Mechanisms of endothelial cell senescence induced by damaging stimuli.
Circulating and endogenous stimuli result in telomeric and non-telomeric DNA damage and alter energy sensor pathways, such as those involving the sirtuins (SIRT1, SIRT3 and SIRT6). Consequently, endothelial cells activate tumour suppressor pathways (tumour antigen p53–cyclin-dependent kinase inhibitor 1A (also known as p21) and retinoblastoma protein (pRb)–cyclin-dependent kinase inhibitor 2A (also known as p16), which reprogrammes endothelial gene expression resulting in a host of cellular and molecular changes. For example, endothelial cells lose their proliferative potential and

become senescent (blue cells); activate the senescence-associated secretory phenotype (SASP), which includes inflammatory pathways such as nuclear factor NF-kappa B (NF-κB) and secretion of inflammatory cytokines and reactive oxygen species (ROS); and attenuate the production of critical vasoactive molecules (e.g. nitric oxide (NO)). Collectively, the cellular and molecular changes promote arterial dysfunction resulting in cardiometabolic diseases. CXCL11, C-X-C motif chemokine 11; DDR, DNA damage response; MMPs, matrix metalloproteinases; PAI-1, plasminogen activator inhibitor 1; TGF-β, transforming growth factor-β.