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Review

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Targeting vascular senescence in cardiovascular disease with aging

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Abstract

Aging is a major risk factor for atherosclerosis and cardiovascular disease (CVD). Two major age-associated arterial phenotypes, endothelial dysfunction and large elastic arterial stiffness, are autonomous predictors of future CVD diagnosis and contribute to the progression of CVD in older adults. Senescent cells lose the capacity to proliferate but remain metabolically active and secrete inflammatory factors termed senescence-associated secretory phenotype (SASP), leading to an increase in inflammation and oxidative stress. Accumulation of senescent cells is linked with the progression of age-related diseases and has been known to play a role in cardiovascular disease. In this brief review, we describe the characteristics and mechanisms of senescent cell accumulation and how senescent cells promote endothelial dysfunction and arterial stiffness. We focus on a range of novel therapeutic strategies aimed at reducing the burden of endothelial dysfunction leading to atherosclerosis through targeting senescent cells. Studies have begun to investigate a specific class of drugs that are able to selectively eliminate senescent cells, termed senolytics, which have shown great promise in reversing the aging phenotype and ameliorating pathologies in age-related disorders, creating a new opportunity for aging research. Generating therapies targeting the elimination of senescent cells would improve health span and increase longevity, making senolytics a promising therapy for cardiovascular diseases.

Keywords: Vascular aging, endothelial dysfunction, senescence, senolytics, cardiovascular disease

INTRODUCTION

Cellular senescence is a hallmark of aging and cardiovascular dysfunction

Cardiovascular disease (CVD) is the leading cause of death worldwide. In America, the cost of cardiovascular disease in the healthcare system has reached over $300 billion per year[1]. Aging is associated with a progressive decline in various physiological processes, leading to increased risk for multiple chronic diseases. Indeed, aging is a major risk factor for CVD, with over 50% of all deaths in Americans aged over 85 due to CVDs[1,2]. Thus, age-related CVD is an important area of study and understanding why age is such a critical component of disease etiology is critical. Although there are many studies relating CVD to aging, the molecular mechanisms underlying the age-associated increase in CVD are still being elucidated and therapies targeting the age-related mechanisms of cardiovascular disease may prove efficacious. Aging has a remarkable impact on the heart and arterial system, leading to cardiac hypertrophy[3], altered left ventricular (LV) diastolic function, increased arterial stiffness and thickening[4], and impaired endothelial function[5-7]. These pathophysiological changes contribute to the increased prevalence of CVDs such as atherosclerosis, hypertension, myocardial infarction, and stroke[8]. Endothelial cells (ECs) line the inside of all blood vessels and are more prominent in the vasculature than any other cardiac cell type, with a probable ratio of ECs to cardiomyocytes being 3:1[9,10]. This indicates an essential role for endothelial cells in cardiovascular health and disease progression. Indeed, ECs participate in many biological processes such as arterial stiffness, angiogenesis, coagulation, and systemic metabolism[11,12], and maintaining EC homeostasis is critical for preventing endothelial dysfunction, atherosclerosis, and CVD.

Cellular senescence, the arrest of cell division in response to the activation of tumor suppressors, contributes to aging and age-related diseases. Senescence is a response mechanism to stress activated by a variety of stimuli, including telomere attrition, hypoxia, oxidative stress, mitochondrial dysfunction, and impaired autophagy[13]. There are two main types of cellular senescence: replicative senescence and stress-induced premature cellular senescence. Replicative senescence occurs through telomere attrition, where telomeres positioned at the end of a chromosome replicate incompletely during cell division. Telomeres play a large role in chromosomal stability and DNA replication, and when telomeres shorten, it leads to DNA damage and induces replicative senescence, mainly through the p53 or p16Ink4a signaling pathways. Stress-induced premature senescence advances through oxidative stress or irradiation, constitutive activation of mitogenic stimuli, oncogenic activation, and metabolic stress. Stress-induced senescence is also facilitated through p53 or p16Ink4a signaling pathways. P53 signaling is predominantly activated through DNA damage and telomere dysfunction, whereas p16Ink4a signaling is mainly associated with mitogenic stress, and overall cellular stress[14,15].

In response to a loss of function, senescence can act as a protective mechanism and can lead to beneficial effects on physiological and pathological processes, comprising wound healing, host immunity, and tumor suppression[13]. However, senescent cells can also have negative effects that are associated with a decline in overall physiological function and health. Senescence causes the loss of repair mechanisms due to cell cycle arrest. At the same time, senescent cells remain metabolically active and produce inflammatory factors, collectively termed senescence-associated secretory phenotype (SASP)[16,17]. With advancing age, cells chronically accumulate damage and can reach a threshold of cellular stress that promotes their withdrawal from the cell cycle, increasing senescent cell accumulation[17]. The discovery of replicative senescence by Hayflick and Moorfield in 1961 led researchers to believe that aging and senescence are causally linked[18]. In a study evaluating SASP as a potential driver of age-related dysfunction, a cohort aged 60-90 years old were assessed. SASP proteins composed of growth differentiation factor 15 (GDF15), TNF receptor superfamily member 6 (FAS), osteopontin (OPN), TNF receptor 1 (TNFR1), ACTIVIN A, chemokine (C-C motif) ligand 3 (CCL3), and IL-15 were positively associated with age, frailty, and adverse surgery outcomes. The combination of these SASP proteins expected unfavorable outcomes significantly better than a single SASP

protein or age alone[19]. Because of the potential of senescent cells to contribute to many aging- and disease-related processes, eliminating senescent cells and attenuating SASP have emerged as possible therapeutic approaches to treat age-related diseases. However, understanding the role of senescence in the endothelium, how this contributes to CVD with aging, and whether senescence in this cell type can be pharmacologically targeted to alleviate CVD requires elucidation.

Drugs that target senescent cells are termed senolytics. The first of their types was identified using a hypothesis-driven, mechanism-based drug discovery approach. Because it was hypothesized that cell survival would be beneficial, these initial senolytics targeted anti-apoptotic, pro-survival pathways[20]. As proteomic and transcriptomic datasets revealed that anti-apoptotic pathways were upregulated in one or more senescent cell populations, these pathways became the target for identification of potential senolytic agents[21,22]. The first senolytic agents that were identified for further investigation were ones that targeted several senescent cell anti-apoptotic pathways (SCAPs), could be administered orally, and were natural products with known safety protocols to facilitate translation from bench to bedside. Dasatinib (D), the SRC/tyrosine kinase inhibitor, was one of the first senolytic agents to be intensively researched, as it has been extensively used since 2006[23]. In combination with dasatinib, the naturally occurring flavonoids quercetin and fisetin, which are present in fruits and other foods, have shown promise in attenuating senescent burden[24]. As this field progresses, key priorities for senolytic research should be the continued identification of both reliable and sensitive biomarkers for senescence, and safe, feasible, and tolerable senolytic agents that can be successfully put into the clinical setting. Here, we present a framework describing the role of senescent cells in the cardiovascular system and in the development of atherosclerosis with aging, focusing on new senolytic approaches to target senescent cells and treat disease.

AGING AND SENESCENCE

Hallmarks of cellular senescence

Cell cycle arrest is a key characteristic of the senescent phenotype, promoted by the activation of p53/p21$^{WAF1/Cip1}$ and the Rb-p16^{INK4A} axes[13]. DNA damage, including irreparable double-stranded breaks, is a common trigger of cell cycle arrest that leads to the activation of the DNA damage response (DDR) pathway[13]. Senescence entry can occur via p53, a tumor suppressor pathway induced in response to DNA damage[15]. P53 provokes the senescence response by increasing the expression of the p21 cyclin-dependent kinase inhibitor (CDKI), leading to cell cycle arrest[25]. P21, in turn, inhibits the phosphorylation and inactivation of the tumor suppressor pRB, which can also be controlled by another CDKI, p16, which has also been reported to be upregulated in senescent cells[15]. Both p21 and p16 are common markers of senescence. DNA damage such as cytoplasmic chromatin fragments and mitochondrial DNA damage are also evident in senescent cells[13]. Telomeric changes, such as telomere shortening and dysfunction, are also associated with senescence. When DNA machinery lacks the ability to replicate telomeric DNA completely, the shortening of telomeres occurs. Therefore, telomerase expression or recombination of telomeric DNA may become impaired, leading to telomere attrition after each round of cell division[26,27]. Telomere dysfunction is identified by telomere dysfunction-induced foci (TIF), i.e., increases in p53 localized to the telomere, which indicate telomere-associated DNA damage.

Another marker of senescence includes the forkhead box O (FOXO) family of proteins is made up of 4 transcription factors sharing the forkhead box domain (FH). FOXOs are tumor suppressors that contribute to the regulation of many functions including cell growth and survival, metabolism, and oxidative stress by regulating the expression of several target genes[28,29]. Oncogenic signaling has been shown to activate cellular senescence as a protective mechanism against cancer; this method has been termed oncogene-induced senescence (OIS)[29]. Both FOXO4 and p53-mediated transcriptional activation of p21 triggers cellular

senescence. Research has shown that inducing senescence *in vitro* increases the expression of FOXO4, with no changes to the other FOXO proteins, signifying an explicit function for FOXO4 in the progression of cellular senescence. Baar *et al.* demonstrated that FOXO4 induces senescence and preserves the viability of senescent cells by suppressing their apoptotic responses. FOXO4 can directly bind to and activate the p53-dependent transcription of the senescence-associated p21 gene[30]. Although the specific mechanisms of FOXO4-dependent p53 activation of p21 transcription are unknown and require more research, the FOXO-p53 axis is a key marker of cellular senescence and may represent a potential therapeutic approach in targeting senescence in age-related diseases.

When a cell enters senescence, it can rewire its metabolic activity, exhibit an enlarged flattened morphology, and show increased β-galactosidase (β-gal) activity, a widely accepted hallmark of senescence[13,31]. Senescence-associated β-gal activity has been shown to be increased in atherosclerotic regions and prominent in vascular and arterial dysfunction[11]. Although senescent cells are no longer able to proliferate or divide, they are still able to produce an array of SASP factors (interleukin-6, interleukin-8, monocyte chemoattractant protein-1, plasminogen-activated inhibitor-1, and many others), contributing to inflammation, dysfunction, and disease[32]. When SASP is chronic, it can trigger paracrine senescence in nearby healthy cells both in culture and in human and mouse models of oncogene-induced senescence[33,34]. Among the primary SASP drivers are the transcription factors, NF-kB, C/EPBbeta, and GATA4; the signaling pathways, mammalian target of rapamycin (mTOR) and p38 mitogen-activated protein kinase (p38MAPK)[35]; and DNA sensors, cGMP-AMP (cGAMP) synthase (cGAS). NF-kB, C/EPBbeta, and GATA4 can activate p53, p21, and p16 signaling pathways in response to DDR (DNA Damage Response), arresting the progression of the cell cycle, inducing cellular senescence and increasing SASP production[36]. Senescent cells can also activate the mTOR pathway, which plays a critical part in regulating cellular metabolism, cell growth, and autophagy in senescent-associated diseases. Sung *et al.* found senescent cells displayed increased activity of mTOR and therefore reduced levels of signal-associated autophagy proteins, and inhibition of mTOR pathway led to significant decreases in senescence and increases in signal-associated autophagy proteins[37]. The findings that inhibition of mTOR activity is linked predominantly to inhibition of cellular senescence and prolonged lifespan in model organisms are well described. mTOR drives senescence through the p53/p21 and p16/pRB pathways and is an important contributor to the progression of senescence and age-related diseases. cGAMP and cGAS activate the adaptor protein STING, leading to an increase of type I interferons and inflammatory cytokines, contributing to the inflammatory response[38]. Senescence cells display activated cGAS-STING pathway transcription levels as well as increased expression of inflammatory cytokine mRNAs. In one study, inhibition of cGAS mitigated DNA damage-induced IL-6 expression and cellular senescence in vascular cells[39]. In mice chronically treated with doxorubicin to induce senescence and cardiotoxicity, the inhibition of endothelial-specific STING significantly prevented doxorubicin-induced cardiotoxicity and endothelial dysfunction[40]. Indeed, the cGAS STING pathway may be a novel therapeutic target for eliminating senescent cells and preventing heart disease. Cell surface proteins have also been identified as SASP regulators in replicative senescence; these include notch1 and dipeptidyl-peptidase 5 (DPP4)[41]. It has been previously shown that senescent cells can communicate with their microenvironment through NOTCH signaling, an increase in reactive oxygen species (ROS), development of cytoplasmic bridges, and excretion of extracellular vesicles such as exosomes[13], which are important characterizations of the senescent secretome. DPP4 has also recently been utilized as an *in vitro* marker of senescence[42]. Other newly identified markers of senescence include PD-L[43], uPAR[44], GPNMB[45], and CD153[46]. The urinary marker α-Klotho was also recently identified as a non-invasive way to measure cellular senescence that may be useful in clinical trials[47]. However, none of these senescence-associated changes/markers are fully sensitive or specific to senescence, and as a result, elucidating the effects of aging, disease, or interventions on senescent cell accumulation or clearance requires the use of multiple markers.

Dysregulated metabolism in senescent cells is associated with dysfunctional mitochondria and impaired mitochondrial dynamics. This mitochondrial dysfunction leads to amplified production of mitochondrial reactive oxygen species (ROS) that can damage macromolecules including proteins, lipids, and DNA[48]. Mitochondrial dysfunction occurs with aging and contributes to vascular endothelial dysfunction, increased arterial stiffness, and cardiovascular disease through increases in mitochondrial ROS[49]. Likewise, mitochondrial ROS has also been shown to trigger DNA damage through the shortening of telomeres in addition to telomere-induced senescence[50]. Senescent cells have dysfunctional mitochondrial respiration and greater mitochondrial superoxide production compared to non-senescent cells[51]. Interventions known to decrease mitochondrial ROS have demonstrated retardation of telomere shortening, leading to improvements in lifespan[48]. Mitochondria in senescent cells become hyperfused and elongated, suggesting mitochondrial dysfunction is an indicator of senescence and a possible target for senescent cell clearance[52]. An elongated and hyperfused mitochondrial network is associated with a reduction in FISI expression, a protein important in mitochondrial fission. Studies have shown that the knockdown of FIS1 has led to an abundance of ROS production and the induction of cellular senescence[53]. However, overexpression of FIS1 prevented mitochondrial elongation and reversed the senescent phenotype[53], suggesting mitochondrial fission may protect against cellular senescence. Mitoquinone (MitoQ) is a highly effective mitochondrial antioxidant. Treatment with MitoQ has been shown to improve arterial stiffness *in vivo*, and *in vitro* evidence suggests MitoQ may also delay the development of senescence[54,55]. Interventions aimed at reducing mitochondrial ROS with MitoQ have been shown to decelerate telomere shortening and extend replicative lifespan[56]. Thus, dysfunctional mitochondria are important factors in senescence accumulation and the development of CVD and other age-related diseases. Research targeting mitochondrial ROS to delay senescence, as well as the senolytic potential of MitoQ, is currently being conducted with promising results.

CARDIOVASCULAR-SPECIFIC IMPLICATIONS OF CELLULAR SENESCENCE AND AGING

Vascular aging, a predictor of cardiovascular diseases such as hypertension, aortic aneurysms, and atherosclerosis[57], is characterized by reduced endothelium-dependent dilation and increased arterial stiffness. Vascular aging is also associated with elevated systolic blood pressure, decreases in diastolic pressure, and increased central venous pressure[4]. These functional and structural changes are associated with endothelial cell (EC) and vascular smooth muscle cell (VSMC) dysfunction, infiltration of immune cells in arterial tissues, and alterations in the extracellular matrix (ECM) of arteries. In addition, aged ECs have reduced production of vasodilators such as NO and prostacyclin and increased production of vasoconstricting factors including endothelin and ROS. Aged VSMCs have disturbed mechanosensing capabilities and adverse changes in ECM structure that contribute to stiffer arteries through increases in collagen and loss or breaks in elastin. Together, these age-related EC and VSMC changes lead to a proinflammatory, pro-thrombotic, proliferative phenotype that contributes to increases in arterial stiffening that compromise arterial compliance and increase the risk of atherosclerosis[58].

Cellular senescence is a fundamental process of cellular aging, promoting a molecular microenvironment with increased inflammation and SASP. Senescence has been described across most cell types in the CV system including cardiomyocytes, endothelial cells, fibroblasts, smooth muscle cells, immune cells, and cardiac progenitor cells. While the drivers of senescence are comparable in most cell types, including telomeric shortening, oxidative stress, mitochondrial dysfunction, and DNA damage[59], the consequences of senescence across cell types can vary. Based on previous studies, senescent endothelial cells appear to be major contributors to the progression and development of CVD, making them a novel target for senolytic therapy and the focus of this review[60].

Endothelial dysfunction

Endothelial cells (EC) make up the inner lining of blood vessels and are directly exposed to endogenous signals and metabolites in the circulatory system. ECs display functional heterogeneity in structure and function. ECs are necessary components of many biological processes, such as vasoreactivity, arterial stiffness, angiogenesis, coagulation, and systemic metabolism. ECs that line the large elastic arteries have important roles in sustaining vascular homeostasis. The main function of the endothelium is to produce vasoactive substances, mainly nitric oxide (NO), to regulate vascular tone. In addition, ECs form a continuous monolayer that acts as a barrier to control substance exchange between the lumen, vascular wall, and parenchyma[61]. This barrier function involves the immunoregulatory recruitment of leukocytes. Upon injury or inflammatory stress, ECs become activated and present adhesive molecules including intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selection at their cell surface. This facilitates the migration of leukocytes to the endothelium, leading to a reactive and proinflammatory endothelial phenotype that is essential for tissue repair in response to injury. However, in a state of chronic inflammation, as with atherosclerosis or advanced age, these inflammatory responses become pathogenic and contribute to endothelial dysfunction that is associated with arterial dysfunction and disease.

Senescence plays a major role in dysfunction in the endothelium, contributing to chronic inflammation and vascular dysfunction[62]. The characteristics of the vascular framework, in addition to the high turnover rate of endothelial cells, likely predispose these cells to senescence. Endothelial cells are one of the primary cell types that become senescent with aging, and multiple studies have shown endothelial cells predominant in vascular beds of multiple organs and tissues with advancing age, contributing to a variety of pathological processes associated with cardiovascular disease. Senescent cells are distinct morphologically and produce a secretory phenotype specific to ECs. Senescent ECs are flat, enlarged and do not change phenotypically or in orientation in response to laminar sheer stress[63]. EC senescence is characterized by an age-associated reduction in EC function, including loss of control over vasodilation, blood coagulation, oxidative stress, inflammation, and immune cell infiltration[64].

Senescent ECs have reduced NO production, which is the key regulator of the endothelium, an increase in ROS, p21Cip1 and p53 expression, and SASP factors, contributing to the development of inflammation and disease[60]. Yokoyama *et al.* demonstrated that increased endothelial p53 is associated with endothelial dysfunction. Utilizing a EC-p53KO mouse model, endothelium-dependent vasodilation and ischemia-induced angiogenesis were significantly attenuated compared to a model of p53 over-expression, making p53 an important target of endothelial function[65]. Senescent EC SASP is heterogeneous and determined by cell type and mechanisms of senescence. Senescent ECs can increase expression of the pro-fibrotic cytokine endothelin-1 and increase expression of proinflammatory adhesion molecules such as VCAM-1 and ICAM-1, contributing to the recruitment of immune cells to the vessel wall and surrounding tissues, further increasing inflammation[66,67]. Senescence accumulates in cells with aging and senescent ECs are associated with endothelial dysfunction, promoting vascular remodeling, atherosclerosis, heart failure, and other age-related diseases[11]. Thus, maintaining EC homeostasis via the targeting of senescent ECs is a promising approach for senolytic therapy.

Atherosclerosis

Atherosclerosis is a chronic CVD that contributes substantial risks to human health. Atherosclerosis underlies many CVDs such as peripheral vascular disease, coronary heart disease, and stroke. Atherosclerosis develops with aging as vascular walls become stiffer, and plaques made up of cholesterol, fat, calcium, and fibrous tissue build up within blood vessels. The pathogenesis of atherosclerosis is multifaceted, involving multiple cell types, including ECs, VSMCs, foam cells, and immune cells.

Endothelial dysfunction is the preliminary step in atherosclerotic progression. In addition to endothelial dysfunction, leukocyte adhesion, foam cell formation, and smooth muscle cell phenotypic transitions are key factors in the progression of atherosclerosis[68]. Endothelial cells can cover atherosclerotic lesions, forming a "cap" that is critical for the protection of plaque stability; however, attrition of the endothelium can lead to plaque instability at the affected lesion, which can contribute to the development of atherothrombosis[69]. Due to ECs' ability to increase expression of VCAM-1 and ICAM-1 and recruit leukocytes, increased endothelial inflammation is thought to be a negative process that aids in maintaining the chronic inflammatory environment of the atherosclerotic wall. Paracrine signaling of senescent ECs to neighboring cells increases senescent cell accumulation, promoting a host of SASP factors, further contributing to disease. Senescent ECs have been shown to accumulate within atherosclerotic lesions and may contribute a causal role in the development of atherosclerosis by contributing to increased ROS and inflammatory cytokines, such as MCP-1 and Il-1B. These factors will, in turn, impair endothelium-dependent vasodilation and perturb glucose metabolism by reducing microvascular perfusion and increasing endothelial inflammation in metabolically active organs[63]. Maintaining endothelial cell homeostasis is important for reducing the risk of atherosclerotic progression and development.

Senescent vascular cells, including ECs, VSMCs, and foam cells, have all been observed in atherosclerotic lesions[70]. VSMCs are critical to atherosclerotic lesion development, and senescence accumulation in these regions may be a driving force for atherogenesis[67]. VSMCs can perform a phenotypic switch and acquire macrophage markers and pro-atherogenic properties[71]. The switching of VSMCs to macrophage-like cells may be promoted through lipid accumulation in the atherosclerotic plaque. Indeed, the increase of cholesterol in VSMCs can activate multiple proinflammatory genes and suppress VSMC marker genes, thus inducing this phenotypic shift. Senescence may also contribute to the phenotypic shift of VSMCs by increasing inflammation and SASP, although more studies are necessary. Furthermore, there is increasing evidence that endothelial and VSMC senescence-associated dysfunction promotes atherosclerotic progression by promoting enlargement of the necrotic core, increasing degeneration of the extracellular matrix (ECM), reducing cap thickness, as well as by promoting erosion, calcification, and intraplaque angiogenesis[72]. Loss of functional VSMC after induction of senescence can promote necrotic core formation and the inefficient clearance of senescent VSMCs leads to secondary necrosis and inflammation[72]. The ECM, a key noncellular component to all organs and tissue, also contributes to atherosclerotic disease progression. Although there is not currently a lot known about the specific interplay between senescent cells and the ECM, proinflammatory factors in senescent cells can promote fibrotic changes, increasing collagen deposition in the ECM which is associated with the progression of atherogenesis[73]. Senescent cells can also degenerate the fibrous cap that usually prevents atherogenic plaque rupture, resulting in plaque instability and clinical consequences such as myocardial infarction and stroke. In studies using pharmacological or transgenic approaches to eliminate senescent cells in the $Ldlr^{-/-}$ mouse model of atherosclerosis, the removal of senescent cells protected deteriorated fibrous caps in atherosclerotic lesions, reduced inflammation, and improved vascular function[74]. Other studies have shown that clearance of vascular cell senescence improved atherosclerotic progression along with other comorbidities in different mouse models of disease[75-77]. These findings indicate that there may be a therapeutic benefit of utilizing senolytic agents in atherosclerosis and reducing morbidity and mortality from CVD.

SENOLYTICS

Targeting senescent endothelial cells to improve vascular function

Senolytics are an emerging class of drugs able to selectively eliminate senescent cells through cell death mechanisms while leaving healthy, non-senescent cells alive. This concept was hypothesized after positive results were found in studies utilizing the INK-ATTAC mouse. In this model, Cdkn2a (encoding for

p16Ink4a) is activated, inducing apoptosis in cells expressing p16Ink4a after treatment with the small molecule (AP20187)[78]. This model demonstrated that the removal of senescent cells improved health span and function in multiple tissues, setting the stage for the important work committed to identifying novel mechanisms and compounds that can selectively ablate senescent cells.

One such endeavor involved the use of another genetic model to identify/track senescent cells. To do so, Demaria *et al.* utilized the 3MR (trimodility reporter) fusion protein mouse and a mouse expressing a senescence-sensitive promoter, i.e., the tumor suppressor p16INK4a[79]. These investigators engineered a bacterial artificial chromosome (BAC) containing the murine p16INK4a locus, driving the 3MR expression. The result was a mouse model that was capable of tracking senescent cells via luminescence, SA-gal staining, and p16INK4a mRNA levels. Importantly, when cellular apoptosis was activated in these p16-3MR mice by exogenous ganciclovir (GCV), p16ink4a senescent cells were eliminated, alleviating atherogenesis and stabilizing plaques in the arterial wall[79]. Models such as this and the INK-ATTAC mouse model, an inducible model for elimination of p16Ink4a positive senescent cells, have provided proof of concept evidence that clearance of senescent cells can improve physiological function and reverse disease, paving the way for the exploration of senescent cells as potential pharmacological, therapeutic targets.

There are various pathways that may be manipulated to eliminate senescent cells by targeting anti-apoptotic mechanisms, termed senescent cell anti-apoptotic pathways (SCAPs). Because SCAPs are necessary for senescent cell viability, senescent survival proteins have been identified and tested through knockdown experiments, leading to the death of senescent but not non-senescent cells[20]. Drugs that have been developed to target SCAPs have seen the most promising results. A variety of senolytic drugs have been identified, but only a few have been researched in CVD. The combination treatment of dasatinib (D), a tyrosine kinase inhibitor, with quercetin (Q), a flavonoid and antioxidant, has proven highly effective in inducing apoptosis in senescent cells *in vitro* across multiple cell types, as well as in multiple animal models. D&Q treatment in human umbilical vein endothelial cells (HUVECs) demonstrated a senolytic effect, alleviating senescence via the inhibition of autocrine and paracrine actions of the SASP[80]. D&Q treatment in nonhuman primates also reduced SASP factors, increased immune function, and improved intestinal barrier function[81]. Zhu *et al.* first established that the pharmacological elimination of senescent cells with a single dose of D&Q could improve vascular endothelial function in aged mice[20]. Chronic senolytic treatment of D&Q in aged mice improved age-related vascular phenotypes such as vasomotor function, increased NO bioavailability, decreased aortic calcification and osteogenic signaling, and reduced chronic hypercholesterolemia[82]. These data indicate that D&Q may be a viable therapeutic intervention for age-related diseases. Presently, D&Q is one of the few senolytic drugs to be studied in a clinical trial setting. Hickson *et al.* found that in patients with diabetic kidney disease, treatment with D&Q reduced adipose tissue and epidermal senescent cell burden by demonstrating a reduction in $p16^{INK4A}$ and $p21^{CIP1}$-expressing cells, a reduced percentage of cells positive for senescence-associated -gal activity in adipocytes and epidermal cells, and a reduction in circulating SASP factors[83]. Quercetin alone has also been researched in numerous human and mouse studies of cardiovascular disease and demonstrated improvements in hypertension, hypolipidemia, hypoglycemia, and atherosclerosis, although measures of senescence were not included[84]. Thus, the combination of D&Q shows great promise as a senolytic treatment, and studying D&Q in the context of cardiovascular disease, both in long-term and short-term interventions, should be further explored.

The BCL-2 family of proteins is acknowledged as a pro-survival regulator of senescence that is also being explored as potential senolytics. These include ABT263 and Navitoclax, which are specific inhibitors of BCL-2 that lead to the induction of apoptosis in senescent cells[22]. Cell culture-based studies have

demonstrated that Navitoclax reduces the viability of senescent HUVECs *in vitro* and treatment of cultured cells with BCL-xl siRNA also produces senolytic effects in HUVECs compared to other cell types[22], suggesting EC specificity of targeting this pathway. The efficacy and hematological toxicity of the recently described BCL-2 inhibitors, fisetin, A1331852, and A1155463, are still being evaluated[24]. In a study in which aged p16-3MR mice were treated with GCV, inducing genetic clearance of senescent cells, or the senolytic ABT263, Clayton *et al.* found that GCV and ABT263 improved endothelial function via increased nitric oxide bioavailability and reduced oxidative stress[85]. In addition, arterial stiffness, measured by pulse wave velocity, was reduced to young levels in old mice treated with GCV and ABT263[85]. Additionally, a reduction in circulating SASP factors in concert with NO signaling was associated with enhanced NO-mediated EDD subsequent to the removal of senescent cells in both treatment groups[85]. Together, the available evidence suggests that cellular senescence and SASP play a role in vascular aging and that senolytic drugs show potential for ameliorating age-related vascular function. Furthermore, all identified BCL-2 inhibitors have been shown to have specific senoltyic effects in ECs compared to other cell types such as preadipocytes and VSMC, selectively eliminating senescent ECs while sparing proliferating ECs[22]. Although more studies investigating BLC-2 inhibitors on other vascular cell types must be performed, the beneficial impact of these EC-targeted senolytics on broad measures of arterial function suggests that endothelial cells are a good target for senolytic treatment.

A more recently defined form of non-apoptotic cell death, ferroptosis, has also been shown to have senolytic potential. Ferroptosis is a form of regulated cell death that relies on iron and is activated by the failure of glutathione-dependent antioxidant defense, resulting in unchecked lipid peroxidation and ultimately resulting in cell death[86]. Glutathione peroxidase 4 (GPX4) is an antioxidant selenoenzyme that can inhibit ferroptosis by quenching lipid peroxidation. Thus, GPX4 plays a vital role in the regulation of the ferroptosis pathway. RSL3 (RAS-selective lethal 3) is a small molecule inhibitor of GPX4 that has demonstrated the ability to induce ferroptotic cell death *in vitro*. Therapeutic effects of ferroptosis inducers (FINS) have received attention in cancer research[87], but information on their role in the context of aging and senescence is deficient. Multiple studies have suggested the possibility of using FINs to selectively eliminate senescence, by demonstrating increased susceptibility to ferroptosis in senescent cells[88,89]. In a model of senescent tubular cells, treatment with RSL3 cleared senescent cells through ferroptosis and an *in vivo* study led to improved organ transplantation of aged mouse kidney[90]. While RSL3 may have off-target effects and is not stable in plasma, various GPX4 inhibitors have been developed and have demonstrated increased specificity to senescent cells *in vitro*[91]. Senescent cells have been reported to show ferroptosis resistance due to increased expression of GPX4, impaired ferritinophagy, upregulated iron uptake, and inactivated iron efflux via ferroportin[89]. However, because of senescent cell's high iron content, they are good candidates for this form of cell death. Drugs that exploit senescent cell's high iron pool such as TRX-CBI could be a safe and effective senolytic approach. TRX-CBI is a tumor-activated prodrug comprised of a trioxolane-based [TRX] sensor of Fe (II) conjugated to a cytotoxic cyclopropylbenzindoline [CBI] payload which demonstrated selective toxicity in Fe (II)-rich cancer cells[92]. In a study that treated primary human endothelial cells with TRX-CBI and FIN56 to induce ferroptosis, senescent cells were selectively eliminated[42]. Thus, ferroptosis may be a novel senolytic pathway that may be utilized to reduce senescent cell accumulation and improve endothelial and vascular function, although this requires further elucidation.

Recent research has also identified that cardiac glycosides (CGs), a group of steroids, exhibit senolytic properties. These compounds bind to and inhibit the plasma membrane Na^+, K^+ ATPase with high selectivity and affinity. The Na^+, K^+ ATPase regulates plasma membrane (PM) potential, and research has shown that inhibition of the subunit ATP1A1 of Na^+, K^+ ATPase enhances PM depolarization in senescent

cells, which is necessary for senolysis. *In vivo* experiments screening different drugs in models of oncogene-induced senescence, conducted by Triana-Martinez *et al.* and Guerrero *et al.*, demonstrated CGs to be a novel class of broad-spectrum senolytics[93,94]. Two identified CGs, digoxin[95] and ouabain[96], reduced senescent cell accumulation, immune infiltration, and inflammation both *in vitro* and *in vivo*. Ouabain and digoxin may induce increases in genetic expression of the proapoptotic BCL-2 family such as NOXA through activation of the JNK, GSK-3, and P38 pathways, partially mediating their senolytic effects, although this complete mechanism remains elusive[94]. The senolytic activity of CGs also indicates beneficial effects on atherosclerosis, pulmonary fibrosis, and anticancer therapies. Several clinical trials involving CGs include promising candidates as a therapeutic application in the context of cancer and aging[97]. However, the long-term effects of this class of senolytics require further study, as oubain may be associated with potential long-term deleterious effects. Thus, further research and clinical trials are necessary to evaluate the safety and efficacy of this class of senolytic in the treatment of other age-related diseases.

Although the elimination of senescent cells has shown promising results in cardiovascular and age-related diseases, examination of potential off-target or unanticipated side effects of senolytic therapy is also needed. Indeed, although a study examining pulmonary arterial hypertension (PAH) demonstrated high lung p16, p21, and γ-H2AX protein levels, as well as increased vascular senescence and DNA damage in patients with PAH, a detrimental effect of senolytic treatment has been described in the pulmonary circulation. For example, in a mouse model of hypoxia, eliminating senescent cells via the senolytics ABT263 or FOXO4-DRI aggravated the severity of monocrotaline-induced pulmonary hypertension[98]. This leads to the possibility that senolytics interventions may worsen pulmonary hemodynamics and strategies targeted at the elimination of senescent cells should consider the potential impact of senescence on the pulmonary system. More studies are necessary to better elucidate pulmonary senescence and the possibility of senescence as a protective mechanism against pulmonary hypertension and progression.

SUMMARY AND FUTURE DIRECTIONS

This review aimed to provide a brief summary of the effects of aging on cardiovascular disease through the accumulation of senescence, highlighting the crucial involvement of vascular cells in the progression of atherosclerosis and other CVDs. We also sought to describe the potential of senolytics to improve vascular function and reduce CVD in aging. Endothelial dysfunction occurs with aging and promotes reductions in NO, increases in ROS, a proinflammatory phenotype, and is associated with an increase in senescent cell accumulation. Understanding how EC senescence influences endothelial dysfunction, atherosclerosis, and CVD is important in the identification/design of novel effective therapeutics. EC senescence is recognized as a contributing factor to endothelial dysfunction and is a major step in the development of atherosclerosis and other CVDs. Evidence suggests that genetic or pharmacological elimination of senescence, specifically in ECs, can attenuate vascular dysfunction and disease in aging through a reduction in the milieu of SASP factors present in senescence. These findings have also improved our understanding of the endothelium's response to aging and how to combat endothelial dysfunction in this setting. Specifically, targeting endothelial cell senescence appears to be a promising strategy for maintaining endothelial functions and improving vascular health.

Preclinical evidence has shown the potential of senolytics for the treatment and prevention of CVD, leading to the investigation of senolytic therapy in the clinical setting. In a preliminary clinical trial investigating the effectiveness of senolytics, a treatment regimen involving a 3-day administration of D&Q per week for 3 weeks was implemented. This study was conducted on 14 patients with idiopathic pulmonary fibrosis and demonstrated high retention and completion rates, indicating the safety of this treatment[99]. Although there were no significant improvements in pulmonary function, treatment with D&Q led to notable

improvements in physical function, as evidenced by increases in 6-min walk distance and 4-min gait speed. Moreover, correlations were observed between enhanced physical function and a reduction in SASP factors[99]. Additionally, preliminary data from a phase 1 clinical trial involving patients with diabetic kidney disease revealed that senolytic therapy with D&Q reduced senescence burden in both adipose and epidermis tissue. This reduction was associated with a decrease in circulating cytokines and matrix metalloproteinases[83]. In a 12-week pilot study of D&Q treatment on individuals with early-stage Alzheimer's disease (AD), there was no significant difference between neuroimaging endpoints and cognitive function. However, results indicated a reduction in cytokine and chemokine levels associated with senescence as well as a trending increase in A42, a biomarker that is inversely related to Alzheimer's disease diagnosis[100]. These studies show promise for the therapeutic potential of senolytic therapy in eliminating senescence, relieving SASP accumulation, and reducing inflammation, although more compounds with appropriate safety, tolerability, and feasibility need to be developed and investigated. Moreover, clinical trials using senolytic therapy in the context of atherosclerosis and cardiovascular disease should be conducted.

Currently, there is not enough research on the use and treatment of senolytic therapy in cardiovascular diseases in the clinical setting. It is unclear if senescent cell clearance, either systemically or in a cell-specific manner, will impact the cardiovascular system, specifically on health span in general. Furthermore, the long-term effects of senescent cell elimination, both systemic and tissue-specific, are not well known. More research must be conducted to answer these questions. While short-term clearance of senescent endothelial and vascular smooth muscle cells improved cardiovascular function and atherosclerosis in preclinical models, further studies are necessary to ensure that the elimination of this cell population has no adverse effects on systemic function, both long-term and short-term. Nevertheless, the potential of senolytics to transform age-related cardiovascular diseases and improve health span is an exciting frontier.

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Authors' contributions

Analyzed the literature and wrote the article: Hall SA
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Availability of data and materials

Data and articles included in this review are available on PUBMED.

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Both authors declared that there are no conflicts of interest.

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Consent for publication

Not applicable.

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