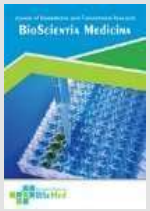
eISSN (Online): 2598-0580



Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: www.bioscmed.com

Senescence-Induced Atherosclerosis: The Potency of Senolytic Therapy

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ARTICLE INFO

Keywords:
Aging
Atherosclerosis
Senescence
Senolytic

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All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/bsm.v8i8.1036

A B S T R A C T

The aging process is an inevitable occurrence that involves physiological changes at the cellular level. The presence of intrinsic and extrinsic stressors can cause cellular damage, leading to senescence and premature aging. Senescent cells undergo activation of the p53/p21 and p16INK4a pathways, induce cell cycle arrest, increased expression of senescence-associated beta-galactosidase (SA-β-Gal), and secretion of SASP (senescence-associated secretory phenotype), leading to "inflamm-aging" or chronic inflammation associated with senescence. These premature aging and "inflamm-aging" accelerates the occurrence of age-related diseases, one of which is atherosclerosis. The relationship between premature aging, senescence, and atherosclerosis has been a focus of research on pathogenesis, prevention, and therapy. Recent research has emphasized the crucial role of senolytics, compounds or agents capable of eliminating senescent cells, in inhibiting the progression of atherosclerosis and slowing down premature aging. Obtaining a more comprehensive understanding of the processes and effectiveness of senolytics in premature aging and atherosclerosis should facilitate the development of more potent medicines to mitigate side effects in the management of cardiovascular disease and extend longevity.

1. Introduction

The world's demographic data indicates a significant increase in the elderly population. Predictions for 2030 show that the number of individuals aged 60 or older will increase by 56% from 2015, and the elderly population above 65 will reach 1.5 billion by 2050, primarily in developing countries.[1] The elderly population is predisposed to chronic diseases, including cardiovascular diseases, stroke, cancer, osteoarthritis, and dementia. At least one chronic condition affects 90% of the elderly in Italy, while two or more chronic diseases affect 60% of elderly people.[2]

The aging process is characterized by a gradual decline in physiological body functions, leading to organ dysfunction and increased mortality risk. These changes become a major risk factor for degenerative diseases.[3] Biological aging is linked to a deterioration in cellular function as an individual grows older. In contrast, premature aging is often linked to the accumulation of DNA damage, accelerated telomere shortening, and the accumulation of Reactive Oxygen Species (ROS).[4,5] Cellular aging or senescence is when a cell no longer undergoes growth or division but does not undergo apoptosis. The accumulation of senescent cells will lead to impaired tissue regeneration, resulting in disease onset.[6]

Coronary heart disease is one of the chronic diseases with the highest mortality rates. Data from the United States in 2020 indicated that 41.2% of

deaths due to cardiovascular diseases were caused by coronary heart disease.[7] The typical etiology of coronary heart disease is atherosclerosis, characterized by the accumulation of lipid deposits on the blood vessel walls, leading to thickening of the intima layer, narrowing of the blood vessel diameter, and impaired blood flow.[8] Aging is one factor that can contribute to the development of atherosclerosis. This is because as people age, their immune cell function tends to deteriorate, a process known as immunosenescence, which is particularly common among the elderly.[9] Senescence cells express the Senescence Associated Secretory Phenotype (SASP), characterized by the release of various cytokines, chemokines, and proteases. SASP affects the cellular microenvironment through paracrine pathways, prompting the release of proinflammatory factors that result in the emergence of persistent and low-grade systemic inflammation known as "inflammaging," leading to damage in the structural component.[10] In the cardiovascular system, SASP affects endothelial cells, vascular smooth muscle cells, and cardiomyocytes and plays a role in the development of atherosclerosis and coronary artery disease.[9]

Given the understanding of the function of senescent cells in age-related ailments, there is potential for medicines that specifically target these cells to become viable therapy choices. These therapies would focus on clearing senescent cells or neutralizing their proinflammatory effects. One action mechanism of senolytics is inducing the apoptosis of senescent cells.[11,12]

Hallmark of senescence

Senescence is a state in which cells permanently stop dividing, or the cell cycle arrest occurs.[3] Nevertheless, the cells continue to exhibit metabolic activity, show resistance to programmed cell death (apoptosis),[13] and have the ability to produce SASP, which is a combination of different signaling molecules, proinflammatory agents, and enzymes capable of breaking down the extracellular matrix.[9]

Cellular senescence should not be equated with aging. The initiation of the senescence program can occur independently of the organism's age. It involves diverse physiological processes beyond aging and is linked to various age-related illnesses.[13] Normal cells can transition into senescent cells due to intrinsic and extrinsic factors. Extrinsic factors, referred to as premature cellular senescence brought on by stress, result from being exposed to various stressors that are genetically harmful, including chemotherapeutic drugs, radiation, UV light, chemical mutagens, and viral infections. Intrinsic factors consist of DNA replicative stress, such as DNA oncogene activation, deficient DNA repair, epigenetics alteration, oxidative stress, and telomere shortening.[14,15] The presence of intrinsic and extrinsic stressors leads to DNA damage and induces the DNA damage response (DDR). DDR is initiated upon detection of DNA lesions by sensor proteins. These sensors include the MRN complex (MRE11-RAD50-NBS1), which recognizes double-strand breaks (DSBs) and other forms of DNA damage. Upon detection of DNA damage, DDR signaling pathways are activated. Key transducer kinases such as ATM (ataxia-telangiectasia mutated), ATR (ATM and Rad3-related), and DNA-PK (DNA-dependent protein kinase) play crucial roles in amplifying the damage signal. In cases where DNA damage is irreparable, persistent DDR signaling leads to the establishment of cellular senescence.[16] Following the onset of senescence, cells display a variety of changes in molecules and cells, which include enlarged cells, irregular cell morphology, elevated lysosomal content, mitochondrial accumulation, enlarged nucleus, and heightened DNA damage.[15]

Cycle cell arrest

The cycle cell arrest of senescent cells is initiated through the transcription factors p16/Rb and p21/p53. These proteins inhibit the Cyclin-Dependent Kinase (CDK) enzymes necessary to initiate specific stages in the cell cycle. By inhibiting CDK, the cycle of cells is stopped in the G1 phase.[17,18] In the p16/RB pathway, the protein p16 is activated by the

INK4a/ARF genetic locus, inhibiting the binding of the CDK4-CyclinD complex. Meanwhile, DNA damage triggers the activation of the P53/P21 protein. Phosphorylation of p53/p21 inhibits the binding of the CDK2-CyclinE complex. Both pathways converge on hypophosphorylated RB, stabilizing its binding with E2F. Under normal conditions, when RB is phosphorylated by CDK, the RB-E2F binding weakens, allowing E2F to dissociate and role the function as a transcription factor. When CDK is inhibited by p16/RB or p53/p21, hypophosphorylated RB occurs, stabilizing the RB-E2F binding, preventing E2F from binding to its promoter, preventing gene replication from stopping in the G1 phase and going into the S phase of the cell cycle.[19,20]

Evading apoptosis

Apoptosis is triggered by either internal or external impulses, and it is necessary to maintain tissue integrity and homeostasis. Its dysregulation is associated with the onset of diverse diseases. Given its pivotal role in normal cellular turnover and tissue balance, aberrant apoptosis during aging increasingly adds to the pathogenesis of various illnesses associated with aging.[21] Senescent cells exhibit resistance to apoptosis varied mechanisms contingent upon cell type and nature of apoptotic triggers[22]. The B-cell lymphoma 2 (Bcl-2) family proteins are recognized as critical regulators of the intrinsic deceased pathway. Antiapoptotic proteins impede apoptosis initiation, whereas proapoptotic proteins stimulate controlled cell death. Consequently, the existence or nonexistence of specific proteins significantly influences cell survival and susceptibility to apoptotic stimuli. Under normal conditions, the Bcl-2 protein is known as an apoptosis inhibitor. Reducing anti-apoptotic BCL-2 levels activates BAX (BCL-2 Associated X protein) and BAK (BCL-2 Antagonist/Killer). Activation of BAX and BAK leads to their oligomerization and development of pores, leading to Mitochondrial Outer Membrane Permeabilization (MOMP) and initial release of Cytochrome-C from mitochondria, which initiates a cascade of reactions culminating in apoptosis cell. Senescent cells have an upregulation of anti-apoptotic proteins such as Bcl-2, Bcl-XL, Bcl-W, and Mcl-1. That will inhibit the activation of BAX and BAK by impeding the formation of the apoptosome and the release of cytochrome C from mitochondria; this mechanism enables cells to evade apoptosis.[23–25]

Overexpressed Sa-β-Gal

Another characteristic of senescent cells is the heightened activity of senescence-associated β-galactosidase—beta-galactosidase functions as a lysosomal hydrolase enzyme. Elevated levels of this enzyme can lead to macromolecular proteoglycans' breakdown and basement membranes' disruption by hydrolyzing glycosidic bonds, causing the separation of amino polysaccharide side chains from core proteins[26]. Lysosomal β-galactosidase activity is typically observed in most mammalian cells at pH 4. Nevertheless, β-galactosidase activity can be observed at pH 6 in senescent cells, which serves as an indication of cellular senescence. Senescent cells undergo increased lysosomal biogenesis in response to the accumulation of damaged proteins and organelles requiring degradation. This upsurge in lysosomal numbers consequently leads to heightened levels of lysosomal enzymes, including beta-galactosidase. The gene that encodes lysosomal beta-galactosidase, GLB1, is upregulated in senescent cells. The upregulation of GLB1 likely responds to the altered metabolic and stress conditions within senescent cells, necessitating enhanced lysosomal activity to preserve cellular homeostasis.[26,27]

Senescence-associated secretory phenotype (SASP)

The term SASP refers to a complex secretion profile generated by cells undergoing senescence. The recognized categorization of SASP components includes signaling factors, proteases, extracellular matrix (ECM) proteins, and non-protein constituents. Based on their molecular mode of action, these components encompass receptor-interacting entities such as soluble signaling molecules like cytokines,

chemokines, and growth factors; molecules that act directly like non-protein elements, ROS, and nitric oxide; and regulatory factors such as matrix metalloprotease inhibitors and plasminogen activator inhibitors.[9] The composition and potency of SASP exhibit considerable variability, contingent upon factors including the trigger and length of time of stressor, environmental cues, and type of cell.[28]

The Senescence-Associated Secretory Phenotype (SASP) undergoes initial regulation mainly at the transcriptional level. The two main transcription factors, C/EBP and NF-κB, facilitate the activation of SASP in response to triggers inducing senescence.[29] SASP entails a multifaceted secretion profile comprising inflammatory cytokines, chemokines, growth factors, and proteases. The activation and control of SASP entail engagement with numerous signaling pathways, notably NF-κB, C/EBPβ, JAK/STAT, and p38 MAPK. These pathways modulate SASP constituents, impacting the local tissue milieu and systemic responses. NF-κB is a major controller of both inflammation and cell viability and is crucial in SASP activation. In senescent cells, activation of NF-κB takes place as a response to DNA damage, reactive oxygen species (ROS) accumulation, and other factors promoting senescence. Once activated, IκB kinase (IKK) phosphorylates IκB, a substance that inhibits NF-κB, leading to its phosphorylation causes the breakdown of IκB and its subsequent release. As a result, NF-κB is able to go into the nucleus. When activated, NF-κB translocates to the nucleus, where it binds to promoters of various SASP genes, instigating their transcription. NF-κB governs the production of a wide range of SASP factors, including pro-inflammatory IL-6, IL-8, and Matrix Metalloproteinases (MMPs), which significantly influence the tissue microenvironment, potentially fostering both paracrine senescence and immune surveillance of senescent cells.[30,31]

C/EBPβ (CCAAT/Enhancer-Binding Protein Beta) is a transcription factor that plays a crucial role in the regulation of SASP. It operates independently and in collaboration with NF-κB to enhance the manifestation of SASP elements. C/EBPβ is recruited to Senescence-Activated Enhancers (SAEs), which catalyzes the transcription of neighboring SASP genes. The functionality of C/EBPβ is contingent upon its phosphorylation status, which impacts its capacity to bind to DNA and govern transcription. Activation of C/EBPβ triggers the elevation of various chemokines and growth factors, thereby contributing to the pro-inflammatory environment characteristic of SASP.[32–34] The other pathway, JAK/STAT3, regulates various proinflammatory cytokines. Elevated phosphorylation levels of JAK1/2 and STAT3 are observed within senescence and aged tissues.[35]

Senescence not only influences intracellular events but also extends its impact to the extracellular milieu, facilitating communication with neighboring cells through the secretion of a diverse combination of factors that can influence and modulate the responses of neighboring cells that are not undergoing senescence. SASP assumes a pivotal role in mediating various pathophysiological effects associated with senescent cells, contributing to harmful conditions such as long-term inflammation, tissue remodeling, along potential cancer progression.[36,37]

Microenvironment alterations

Through SASP, senescent cells can induce chronic inflammation. SASP will continuously secrete pro-inflammatory cytokines such as IL-1α, IL-1β, IL-6, and IL-8, forming low-grade chronic inflammation, termed “inflammaging.” Inflamm-aging refers to persistent low-grade inflammation that typically accompanies aging. This is a systemic inflammation that occurs without the presence of an infection. It is characterized by a subtle but chronic increase in pro-inflammatory markers in the body, which can lead to various age-related diseases such as neurodegenerative disease, atherosclerosis, heart disease, type II diabetes, and cancer.[38,39] Activation of this long-term inflammation will trigger "immunosenescence," which refers to the functional deterioration of the adaptive immune system mainly due to the exhaustion of naïve T cells and excessive proliferation among T and B cells.[13,40]

When inflammation persists over an extended period, it can adversely affect health, and may also increase the risk of premature mortality.[41]

SASP can also induce nearby normal cells to undergo senescence through paracrine signaling. Consequently, these normal cells undergo early aging and lose their ability to regenerate.[42] Senescent cells tend to build up in different tissues and organs due to their resistance to apoptosis. This condition impairs tissue regeneration and organ function by occupying space, disrupting normal cell proliferation, and inducing exhaustion of stem cell pools required for tissue repair and renewal.[43]

Matrix metalloproteinases (MMPs), vital constituents of the senescence-associated secretory phenotype (SASP) released by senescent cells, regulate its influence on the microenvironment. MMPs such as MMP-1, MMP-3, MMP-8, MMP-9, and MMP-13 degrade the extracellular matrix, perturbing tissue architecture and microenvironments, thereby compromising tissue function and exacerbating age-related conditions such as osteoarthritis, cardiovascular diseases, and neurodegeneration through sustained inflammation and tissue injury.[44,45]

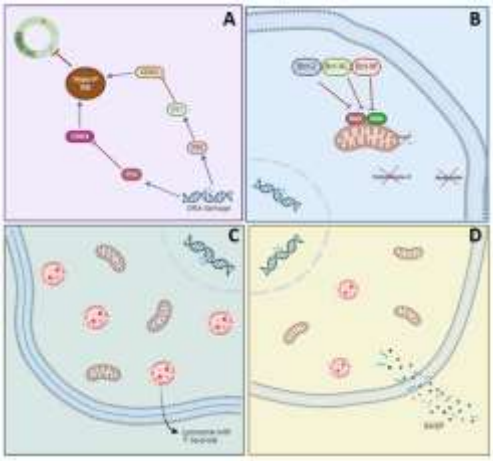

Figure 1. The hallmark of the senescent cell. (A) Cell cycle arrest. The transcription factor p16/Rb inhibits CDK4, while p21/p53 inhibits CDK2, leading to hypophosphorylation of Rb and inhibition of the cell cycle from entering the S phase. (B) Evading apoptosis. Bcl-2, Bcl-XL and Bcl-W inhibit the activation of BAX and BAK, preventing the formation of MOMP, Cytochrome-C, and apoptosis. (C) Overexpression of Sa-β-Gal. Upregulation of GLB1 in response to metabolic changes increased lysosome mass and activity as a response of senescent cells. (D) The secretion of SASP consists of cytokines, chemokines, growth factors, proteolytic enzymes, and regulatory factors.

Senescence and atherosclerosis

Aging induces a cascade of structural and functional modifications in arteries, profoundly influencing their integrity and performance. Notably, there is a notable rise in the ratio of intima to media thickness, a parameter reflecting the relative thickness of the inner layers of the arterial wall. Research indicates a substantial increase in this ratio, ranging from 2- to 3-fold, as individuals transition from early adulthood to advanced age, underscoring the continuous remodeling process of arteries over the lifespan. Furthermore, aging prompts a transition of vascular smooth muscle cells, contributing to an increase in the thickness of the innermost layer of the blood vessel wall. This modification is strongly linked to increased permeability of the arteries, which promotes the development of atherosclerotic disease.[46,47]

Atherosclerosis is not merely a degenerative condition but rather a chronic inflammatory disease marked by the buildup of plaques in the arterial walls. The plaques consist of lipids, cholesterol, and other circulating substances. With time, this accumulation results in the thickening and stiffening of arterial

walls, potentially impeding blood flow and contributing to diverse cardiovascular disorders, including myocardial infarctions and strokes. Atherosclerosis appears to represent a persistent inflammatory state.[48,49]

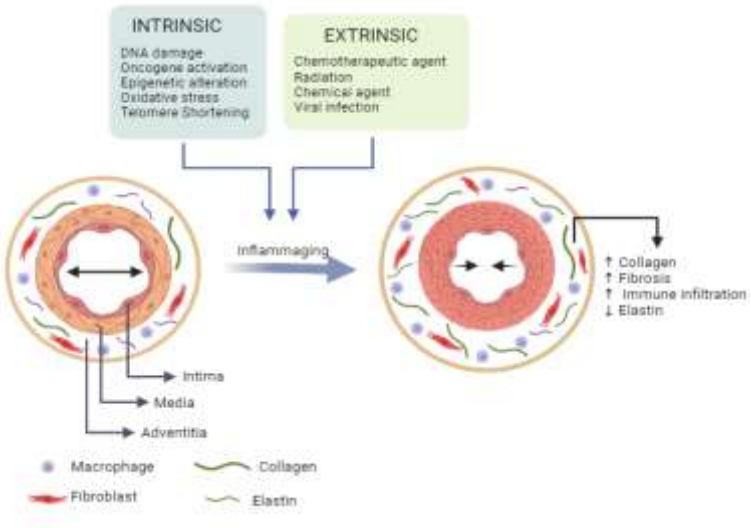

Figure 2. Intrinsic and extrinsic stressors lead to inflammaging and senescence in vascular cells. This results in the thickening of the media layer, and any changes such as increased collagen, increased fibrosis, and decreased elastin influence the stiffening of blood vessels, forming the basis for atherosclerosis.

Atherosclerosis occurs as a result of damage to the endothelium, which leads to the buildup of cholesterol-containing particles that are susceptible to oxidation in the walls of arteries. This process also triggers a continuous inflammatory response. The initiation of both innate and adaptive immune responses contributes to the advancement of atherogenesis, beginning with the initial impairment of the endothelium and culminating in the occurrence of sudden thrombotic problems caused by the rupture or erosion of plaques. Monocytes that enter the inner layer of the artery wall transform into macrophages, which then change into foam cells within the lipid-filled dead tissue at the center of the atheroma. The crystallized cholesterol and other damage-associated molecular patterns (DAMPs) found in atherosclerotic lesions stimulate inflammasomes in macrophages. This stimulation leads to secretion of pro-inflammatory cytokines, such as IL-1, IL-18, and chemotactic factors for T-cells and B-cells. Severe atherosclerosis is characterized by heightened apoptosis and the buildup formation of senescent cells. This creates an environtment of inflammation and promotesthe development of a necrotic core, which eventually causes the rupture of the plaque, the production of blood clots, and acute damage to blood vessels.[41,50,51]

Endothelial cells senescence

In comparison to youthful endothelial cells (ECs), senescence in endothelial cells exhibits various structural and functional alterations. Senescent EC facilitates pathways involved in arterial remodeling, resulting in endothelial dysfunction and arterial stiffening, ultimately triggering the advancement of senescence and atherosclerosis. The attachment of enlarged senescent endothelial cells to the basal membrane is heightened, leading to compromised alignment under laminar shear stress, yet showing resilience against loss of endothelial integrity.[52,53] The hemodynamic conditions within blood flow, blood vessels, and various cardiovascular risk factors (obesity, smoking, hyperlipidemia, and diabetes mellitus) can predominantly induce endothelial

senescence through mechanical and oxidative stress[53]. Exposure to multiple stressors triggers a DNA damage response, activating the p53–p21 pathway, ultimately leading to replicative senescence. The p21 protein governs cell cycle advancement and apoptosis in mature ECs while modulating the magnitude and frequency of hematopoietic precursor cell populations[9]. It was observed that p53 interrupts endothelium-dependent vasodilation, which is crucial for maintaining regular blood flow. Studies have demonstrated that p53 facilitates the angiotensin II (Ang II)-induced deterioration of vasodilation and inhibits eNOS activity by suppressing its phosphorylation at Ser1177.[54,55]

The accumulation of senescent endothelial cells leads to dysfunction in the endothelial barrier, increased levels of inflammatory cytokines (IL-1β, IL-6, IL-8), chemokines (CXCL11, MCP-1), growth factors, and ROS. The secretory profile of senescent cells is influenced by the type, intensity, and duration of exposure to stress-induced aging in endothelial cells. These mechanisms facilitate the infiltration of immune cells while concurrently decreasing the production of vasodilatory Nitric Oxide (NO). Importantly, senescent endothelial cells demonstrate elevated levels of plasminogen activator-1 (PAI-1) and reduced levels of eNOS, thereby elevating the risk of thrombosis and susceptibility to atherosclerosis.[53]

Vascular smooth muscle cell (VSMC) senescence

In blood vessels, the VSMC layer is situated within the tunica media, which comprises multiple layers of VSMCs and elastic fiber layers. Senescent VSMCs undergo irreversible phenotype switching[56]; this switching refers to the ability of VSMCs to change from a 'contractile' phenotype, which is characterized by the expression of specific markers that allow the cells to maintain vascular tone, to a 'synthetic' phenotype, which is associated with increased cell proliferation, migration, and the production of extracellular matrix components. The synthetic phenotype is implicated in the development and progression of atherosclerosis.[57] The contractile phenotype is maintained under normal physiological conditions. It is characterized by the expression of specific contractile proteins such as smooth muscle α-actin (ACTA2), myosin heavy chain (MYH11), and calponin, and contributes to vascular integrity and function.[58] Vascular injury or inflammatory signals often trigger the transition to the synthetic phenotype. Key pathways include PDGF-BB and TGF-β signaling, which stimulate VSMCs to proliferate and migrate.[56] The differentiation of the contractile VSMC phenotype into a mesenchymal-like phenotype is induced by KLF4, which is one of the Yamanaka transcription factors.[53,59] Inflammatory cytokines such as TGF-β signaling can induce the transformation of contractile VSMCs into VSMCs resembling myofibroblasts. These myofibroblast-like VSMCs are implicated in processes related to wound healing and fibrosis and in the formation of fibrous caps within atherosclerotic plaques, which are essential for maintaining plaque stability.[56,60] VSMCs can acquire macrophage-like characteristics through pathways influenced by inflammatory cytokines[61,62] and contribute to the inflammatory milieu of atherosclerotic plaques by secreting pro-inflammatory cytokines and chemokines, such as IL-1α, IL-1β, IL-6, TNF-α, MMP-9, and MCP-1, which can recruit additional immune cells to the site, exacerbating the inflammatory response.[63] These cells can accumulate lipids and transform into foam cells, a hallmark of early atherosclerotic lesions. Foam cells are laden with lipids and contribute to the growth and instability of plaques.[64] The secreted MMPs are involved in the breakdown of collagen and elastin, critical components of atherosclerotic plaque fibrous caps. By degrading these structural proteins, MMPs weaken the structural integrity of the fibrous cap, making it more susceptible to rupture.[64,65] The transition from myofibroblast-like VSMCs to an osteogenic phenotype VSMC can be driven by bone morphogenetic protein and is involved in the calcification of vascular tissues, leading to increased arterial stiffness and complications in atherosclerosis.[56]

Senescence as target therapy

Given the knowledge of particular proteins and pathways influencing senescence, there is extensive ongoing research into drug agents that target molecules involved in senescent cells as potential therapeutic interventions.[66] Two therapeutic agents that have been extensively researched are senomorphic and senolytic. Senolytic drugs are agents designed to induce apoptosis in senescent cells selectively. These drugs target specific pathways that allow senescent cells to resist apoptosis, thereby helping to clear these cells from the body. The removal of senescent cells has improved tissue function and delayed the onset of age-related pathologies in preclinical models.[67]

Xenomorphic drugs, in contrast to senolytics, do not kill senescent cells but rather suppress the harmful aspects of the SASP. By modulating the inflammatory environment created by SASP, these drugs aim to mitigate the detrimental effects of senescent cells on tissue function.[18] Targeting cellular senescence through analytic and xenomorphic drugs presents a promising therapeutic avenue for addressing the complexities of aging and associated disorders. Ongoing research and clinical trials will be crucial in translating these findings into effective and safe therapies.[18,66]

Potency of senolytic

Senolytics are a class of drugs that selectively eliminate senescent cells and show potential as candidates for preventing and treating chronic ailments,[68] including small molecules, peptides, and antibodies.[69] Senolytics selectively induce apoptosis in senescent cells while leaving non-senescent cells relatively unaffected. This is achieved by targeting pro-survival pathways or surface markers specific to senescent cells.[70] By eliminating accumulated senescent cells from tissues, senolytics have shown promise in alleviating or delaying various age-related diseases and extending the health span in preclinical studies.[71]

Navitoclax, also known as ABT-263, is an experimental orally active anti-cancer drug with potential in other therapeutic areas, including the treatment of atherosclerosis through its senolytic effects.[72] Navitoclax targets the Bcl-2 family of proteins, including Bcl-2, Bcl-xL, and Bcl-w, which are major negative regulators of apoptosis. By inhibiting these proteins, navitoclax promotes the apoptosis of senescent cells.[73] Navitoclax binds to the anti-apoptotic proteins Bcl-2, Bcl-xL, and Bcl-w. Through this inhibition, the release of pro-apoptotic proteins occurs. Once these pro-apoptotic proteins are released, they can initiate the translocation of BAX to the mitochondria. This translocation results in mitochondrial outer membrane permeabilization, a critical step in activating the caspase cascade, ultimately leading to cell death.[74] In atherosclerotic Ldlr knockout mice, administration of navitoclax following the establishment of senescence reduces the number of senescent cells, decreases plaque burden, reduces plaque number, and diminishes the average plaque size. Additionally, this treatment correlates with decreased factors involved in plaque formation, including MCP1, IL-1α, TNFα, and the leukocyte receptor vascular cell adhesion protein 1.[75,76] However, other studies state that treatment with ABT-263 resulted in multiple detrimental changes, including increased mortality in atherosclerotic mice. It reduced the number of smooth muscle cells within lesions, potentially due to reduced fibrous cap thickness, a marker of plaque instability essential for maintaining plaque stability.[77]

Dasatinib is a tyrosine kinase inhibitor originally developed as an anti-cancer drug, specifically for treating certain types of leukemia in adults and children. On the other hand, quercetin is a naturally occurring flavonoid found in many fruits and vegetables. When Dasatinib and Quercetin are combined, they form a senolytic therapy.[78] Dasatinib is a tyrosine kinase inhibitor that targets various pathways involved in cell survival and proliferation, while quercetin inhibits several anti-apoptotic proteins such as BCL-XL and HIF-1α. This combination leads

to the induction of apoptosis in senescent cells.[79,80] Treatment involving Dasatinib and Quercetin reduced senescence burden and plaque calcification despite the absence of any observed alteration in plaque size.[80]

When employed in cancer therapy, these medications frequently manifest undesirable side effects including nausea, vomiting, diarrhea, and skin rashes; notably, Bcl-XL inhibitors provoke severe thrombocytopenia. In order to mitigate these consequences, it may be necessary to utilize localized administration by percutaneous intervention to specifically address senescence in a cardiovascular disease context. This could be integrated with senescent cell-targeted delivery techniques, such as the use of galactose-encapsulated medicines, which have been shown to decrease thrombocytopenia following the administration of Navitoclax in a controlled experimental environment. Alternatively, the medications could be administered intermittently, with extended periods of not taking the drugs.[81]

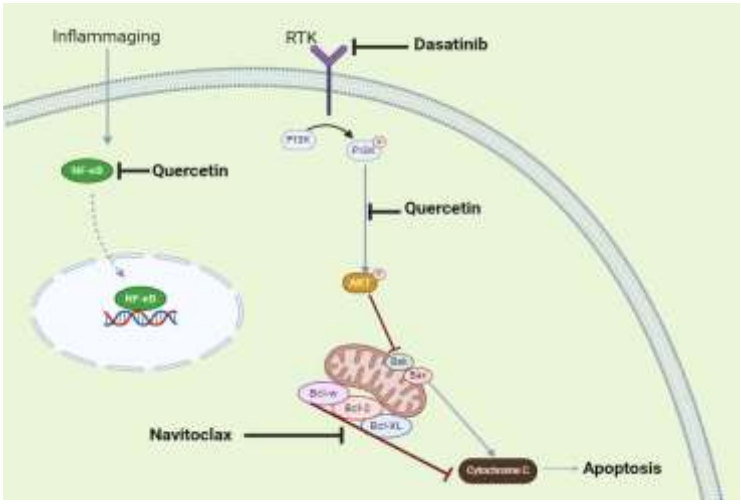

Figure 3. Senolytic mechanism. Navitoclax inhibits the function of Bcl-2 family proteins, activating Bax and Bak and releasing Cytochrome-C, which induces apoptosis. Dasatinib inhibits RTKs, disrupting the PI3K/Akt pathway, which leads to reduced Akt-mediated inhibition of Bax and Bak, consequently triggering the release of cytochrome-C from the mitochondria and initiating apoptosis. Quercetin has been shown to inhibit AKT activation, reducing AKT inhibition towards the activation of Bax and Bak. Additionally, quercetin can inhibit NF-kB activation to migrate into the nucleus, leading to a decrease in gene expression associated with the secretion of pro-inflammatory cytokines related to senescence and affecting cell death. RTK: Receptor Tyrosine Kinase, PI3K : Phosphoinositidine 3-Kinase, Akt (PKB) : Protein Kinase-B, Bcl : B-Cell Lymphoma, NF-κB : Nuclear Factor-Kappa B.

Other senolytic agents have been examined, although not specifically for atherosclerosis. Reut Yosef investigated the effectiveness of the ABT-737 senolytic agent in simulating lung fibrosis, mainly targeting Bcl-XL and Bcl-W.[82] Yi Zhu studied the capability of Fisetin to trigger apoptosis in HUVECs by focusing on blocking the PI3K/AKT pathway.[83] Fisetin has also demonstrated its ability to lower mortality rates and diminish cellular senescence and inflammatory markers in elderly mice infected with SARS-CoV-2.[84] Another senolytic, FOXO4-DRI, has been researched, targeting inhibition in the FOXO4-P53 gene pathways.[85] Given the effectiveness of senolytics in diminishing cellular senescence in various conditions, there is potential for this approach also to yield positive results in managing atherosclerosis.

Table 1. Senolytic agents and molecular targets.

No	Senolytic agents	Target molecular	Ongoing trial	Reference
1	Dasatinib	Tyrosine kinase receptor	Phase 1 (D+Q)	67
2	Quercetin	Bcl-2 family (Bcl-2, Bcl-XL, Bcl-w), PI3K/AKT pathway	Phase 1 (D+Q)	67
3	Navitoclax (ABT-263)	Bcl-2 family (Bcl-2, Bcl-XL, Bcl-w)	In Vivo	76
4	ABT-737	Bcl-W and Bcl-XL	In Vitro	82
5	Fisetin	PI3K/AKT pathway	In Vitro	83
6	Foxo4-DRI	Foxo4-p53 signaling	In Vitro	85

In various experiments conducted on a range of diseases using murine models demonstrated to reduce the number of senescent cells and restore regeneration of the kidneys, leading to increased tubular proliferation, improved function, and reduced fibrosis after injury. Dasatinib and quercetin have been found to reduce senescent markers and improve exercise endurance in mice. FOXO4-DRI has been shown to trigger selective apoptosis in senescent cells induced by aging and chemotherapy, resulting in improved mouse health, including protection of renal structure and function. ABT737 has been shown to eliminate p16-positive cells and suppress hyperplasia in mice with induced skin lesions.[86]

Developing effective and safe senolytics is challenging due to several factors. One primary challenge is ensuring the selectivity and specificity of senolytics, targeting senescent cells without affecting healthy cells. Current senolytics have modest selectivity and may have cell-type-specific effects, complicating their development and application. Additionally, there is a risk of off-target toxicities, where senolytics may impact non-senescent cells, leading to unintended side effects. For instance, dasatinib and quercetin, while effective in reducing senescent cells, may also affect other cell types. Safety and toxicity are significant concerns with senolytics. Navitoclax can have adverse effects such as thrombocytopenia and neutropenia, limiting their clinical use. Determining the optimal dosing regimen is crucial, with intermittent administration generally preferred to avoid impairing tissue repair. Continuous dosing may lead to adverse effects on healing processes. However, finding the balance between efficacy and safety remains a challenge.[87,88]

2. Conclusion

Despite the promising potential of senolytics, there are still limitations and unanswered questions that researchers are actively addressing. One area of focus is the development of more specific senolytic agents that can selectively target senescent cells without affecting healthy tissue. Additionally, the long-term effects of senolytic therapy on overall aging processes and longevity require further investigation.

In conclusion, while senolytics represent an exciting and novel approach to addressing atherosclerotic senescence, further research and clinical trials are needed to understand their benefits, limitations, and long-term effects fully. It's an area of active and evolving research that holds promise for the future of cardiovascular health and aging interventions.

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