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The Aging of Cells: Cellular Senescence and Implications for Age-Related Diseases

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ABSTRACT

Cellular senescence, the aging of cells, is a multifaceted process characterized by the progressive accumulation of molecular and cellular changes. Senescent cells, marked by their proinflammatory secretome called the senescence-associated secretory phenotype (SASP), contribute to tissue dysfunction and promote aging processes. In this review, we provide a comprehensive overview of the current understanding of cellular senescence, with a particular focus on the underlying mechanisms driving cell aging and the strategies of targeting senescent cells to extend healthspan and alleviate age-related diseases. We discuss how factors such as telomere shortening, DNA damage, oxidative stress, and oncogene activation contribute to cellular aging and the development of age-related diseases. Furthermore, we explore the potential senotherapeutic interventions, including senolytic and senomorphic therapies, that target senescent cells. Lastly, we highlight the opportunities and challenges of senotherapeutics in the context of aging and aging-associated diseases, underscoring their potential implications for human health and longevity.

Introduction

Cell aging, also known as cellular senescence, is a state of permanent growth arrest in the cell cycle by loss of proliferative capacity, which has been directly implicated as a key driver of aging and age-related diseases (Di Micco, Krizhanovsky, Baker, & di Fagagna, 2021; Huang, Hickson, Eirin, Kirkland, & Lerman, 2022). This phenomenon was first described in vitro in 1961 by Hayflick and Moorhead using human fibroblasts that entered an irreversible growth arrest known as replicative senescence after 40–60 population doublings (Hayflick & Moorhead, 1961). In addition to telomeric shortening in replicative senescence, cellular senescence occurs in response to cell damages by endogenous and exogenous stressors, including DNA damage, epigenetic changes, reactive metabolites, oxidative stress, oncogene activation, and viral infections, etc. Although cellular senescence is a natural protective mechanism that prevents damaged cells from proliferating and potentially becoming cancerous, it can also contribute to tissue and organ dysfunction in the context of aging and age-related diseases. Therefore, understanding the mechanisms of cell aging and developing potential interventions to slow down or reverse cellular senescence can promote healthy aging and prevent age-related diseases, having significant implications for human health and longevity.

Although senescent cells are in a state of irreversible cell cycle arrest, they are still metabolically active, secreting various bioactive molecules, collectively known as the senescence-associated secretory phenotype (SASP) (Vizioli et al., 2020). SASP is an important feature of senescent cells comprising the release of numerous proinflammatory cytokines, chemokines, growth factors, and proteases. These SASP factors can have both beneficial and detrimental effects. On one hand, they contribute to tissue repair and remodeling by promoting immune responses, attracting immune cells to the site of damage, and facilitating the removal of damaged cells. On the other hand, the SASP can also induce senescence in neighboring as well as remote healthy cells through a paracrine effect. Although paracrine senescence can be a protective mechanism to prevent the spread of cell damage

or compromised genomic integrity, the prolonged accumulation of senescent cells in tissues and organs can also lead to chronic inflammation, immune deficit, stem cell exhaustion, and tumor development.

It is a natural process that senescent cells are eliminated from the body by the immune system. This process involves the recognition of senescent cells by immune cells, such as natural killer cells, macrophages, and T cells, which then induce apoptosis or phagocytosis of senescent cells (Sagiv & Krizhanovsky, 2013). However, the immune system declines with age, and becomes less efficient at clearing senescent cells, leading to aging and age-related diseases by the accumulation of senescence. Therefore, targeting senescence cells by eliminating them (senolytics) or modulating their phenotype (senomorphics) holds great promise as therapeutic interventions to increase healthy lifespan (Zhang et al., 2022).

In this review, we provide a comprehensive overview of the current understanding of cell aging, with a particular focus on the mechanisms and hallmarks of cellular senescence. We then discuss emerging opportunity to intervene through senolytic and senomorphic therapies in ageing and ageing-associated diseases.

Cellular Senescence

Senescent cells, exhibiting irreversible cell cycle arrest while remaining viable, accumulate with age and at pathogenic sites throughout the lifespan. Many senescence-inducing stressors activate signaling pathways such as DNA damage response and the p53/p21$^{CIP1/WAF1}$ or p16^{INK4a}/retinoblastoma protein pathways, resulting in cell cycle arrest and the development of a SASP. Senescent cells have increased resistance to apoptotic cell death even on exogenous stress exposure, owing to upregulation of cell survival pathways, including SRC kinases, the PI3K–AKT signaling pathway, heat shock protein (HSP) pathways, or the BCL-2 family of anti-apoptotic proteins (Zhang, Pitcher, Prahalad, Niedernhofer, & Robbins, 2023). Through upregulation of such pro-survival and antiapoptotic pathways, those senescent cells can survive, despite the cytotoxic microenvironment they create with a proapoptotic SASP.

DNA damage response

The principal trigger of senescent stress is DNA damage, mainly in the form of DNA double-strand breaks (DSBs), which initiates the activation of the DNA damage response (DDR) pathway and the p53/p21 cascade. p21, also known as cyclin-dependent kinase (CDK) inhibitor 1, plays a critical role by inhibiting cyclin-dependent kinase complexes, thereby leading to the repression of cell-cycle genes (Di Micco et al., 2021; Fumagalli, Rossiello, Mondello, & di Fagagna, 2014; Huang et al., 2022). Conversely, senescence induced by epigenetic alterations predominantly operates through the p16/retinoblastoma pathway. Through its inhibitory action on cyclin D-CDK4/6 complexes, p16 prevents the phosphorylation of RB and promotes the formation of the RB-E2F complex, consequently inhibiting the transcription of cell-cycle genes. Accumulating evidence suggests that p21 activation occurs primarily during the early stages of senescence, while p16 contributes to the maintenance of cellular senescence.

Telomere shortening

Telomere shortening stands as one of the earliest and most extensively studied mechanisms underlying cellular senescence. Telomeres, the protective caps at the ends of chromosomes, undergo gradual attrition with each round of DNA replication, primarily due to the inability of the standard DNA replication machinery to fully replicate chromosomal ends. As telomeres progressively shorten, they eventually reach a critical length where telomere-capping factors and protective structures are lost. This critical shortening triggers a DDR similar to that activated by DSBs (di Fagagna et al., 2003). This mechanism acts as a safeguard to prevent cells with damaged or unstable genomes from proliferating and potentially giving rise to harmful mutations or genomic instability. Telomere

shortening is closely associated with replicative senescence, a form of senescence that limits the proliferative capacity of cells.

Oxidative stress

Reactive oxygen species (ROS) and oxidative stress play a pivotal role in the induction of cellular senescence (Ogrunc et al., 2014). ROS are highly reactive molecules generated as byproducts of cellular metabolism, and their levels can increase under various stress conditions, including aging and exposure to environmental factors. While ROS are known to cause DNA damage, they also act as signaling molecules that regulate cellular processes. During senescence, there is an accumulation of ROS due to various factors, including mitochondrial dysfunction, inflammation, and activation of oncogenes. Elevated ROS levels contribute to the initiation and progression of senescence by promoting DNA damage, telomere shortening, and activation of DNA damage response pathways. ROS can directly damage DNA, proteins, and lipids, leading to cellular dysfunction and the activation of senescence-associated signaling pathways.

Mitochondrial dysfunction

Elevated oxidative stress and the accumulation of dysfunctional mitochondria have been closely linked to each other and emerged as a notable hallmark of senescent cells ((Correia-Melo et al., 2016). Specifically, senescence is accompanied by changes in mitochondrial mass, membrane potential, and morphology, indicating mitochondrial dysfunction. Perturbations in mitochondrial function, such as depletion of mitochondrial sirtuins (a class of conserved proteins regulating aging) or selective chemical inhibition of mitochondrial activity, have been shown to induce a distinct form of senescence called mitochondrial dysfunction-associated senescence (MiDAs).

Oncogene activation

Oncogene activation is another potent inducer of cellular senescence. The expression of oncogenes initiates a hyperproliferative phase, which is intricately associated with altered DNA replication and ultimately engages the DDR pathways, leading to the establishment of senescence. This phenomenon, known as oncogene-induced senescence (OIS), represents a crucial safeguard against uncontrolled cell growth ((Di Micco et al., 2006). Moreover, loss of tumor suppressor expression, exemplified by PTEN loss-induced cellular senescence (PICS), has been shown to induce proliferation arrest through mechanisms involving hyperproliferation, DDR activation, and subsequent cellular senescence in vivo. Telomeres are labile to DNA replication stress induced by oncogenes. Accumulation of oncogene-induced telomeric dysfunction leads to the activation of the DDR, as observed in hyperplastic cancer lesions in humans.

Notably, reactive oxygen species (ROS) not only act as DNA-damaging agents but also function as signaling molecules mediating pro-mitogenic effects of oncogenes ((Correia-Melo et al., 2016). The paradoxical role of ROS in promoting both cell proliferation and senescence-associated DNA damage has recently been elucidated. It was unexpectedly discovered that oncogene-induced ROS, generated by NADPH oxidases, contribute to cellular senescence by augmenting the initial hyperproliferative phase associated with altered DNA replication and DNA damage accumulation. This finding provides new insights into the complex interplay between oncogenes, ROS, and cellular senescence, highlighting the multifaceted nature of senescence regulation in oncogenic contexts.

Consequences of Cellular Senescence

Senescent cells are typically eliminated within days to weeks after their formation by immune cells like natural killer cells. However, if the number of senescent cells exceeds a certain threshold, they can accumulate due to a

combination of factors. This may include the proapoptotic senescent cells expressing a SASP that promotes the spread of senescence through paracrine and endocrine signaling, surpassing the immune clearance capacity. Once senescent cell burden exceeds the threshold, the continuous increase in proapoptotic and pro-inflammatory senescent cells can contribute to tissue destruction and organ dysfunction, leading to frailty and age-related disorders. This process can also disrupt immune regulation, further amplifying the accumulation of senescent cells. The persistent accumulation of such senescent cells can lead to a chronic low-grade, sterile inflammatory state known as "inflammaging", and disrupt the function of neighboring and distant non-senescent cells, impairing tissue function and regenerative capacity (Ferrucci & Fabbri, 2018).

Declined physiological function and frailty

As a driver of aging, cellular senescence has been implicated in the development and progression of frailty, a condition characterized by reduced functional capacity, and increased vulnerability to adverse health outcomes in older adults (Bandeen-Roche et al., 2006; Ferrucci & Fabbri, 2018). Frailty is associated with a decline in various physiological systems, including musculoskeletal, cardiovascular, and immune systems. Cellular senescence can contribute to these age-related declines through multiple mechanisms. Senescent cells secrete inflammatory molecules as part of the senescence-associated secretory phenotype (SASP), which can promote chronic inflammation and impair tissue function. This chronic low-grade inflammation can contribute to muscle wasting (sarcopenia), bone loss (osteoporosis), and impaired immune function, all of which are features of frailty.

Furthermore, senescent cells have reduced replicative capacity and altered gene expression, which can interfere with tissue repair and regeneration. This impaired regenerative capacity can lead to the accumulation of damaged tissues and contribute to the functional decline seen in frail individuals. Importantly, the accumulation of senescent cells has been observed in tissues and organs affected by frailty, such as skeletal muscle, cardiovascular system, and immune cells. Senescent cells can disrupt tissue homeostasis, impair the functioning of neighboring cells, and contribute to the overall decline in physiological function associated with frailty.

Age-related diseases

Persistent cellular senescence not only contributes to the process of aging but also plays a causal role in numerous age-related diseases. The accumulation of senescent cells often occurs at pathogenic sites in various major age-related chronic conditions, including Alzheimer's disease, cardiovascular diseases, osteoporosis, diabetes, renal disease, and liver cirrhosis (Bhat et al., 2012; Khosla, Farr, & Kirkland, 2018; Song, Zhao, & Zou, 2020; Zhou et al., 2020). The detrimental effects of senescent cells in age-related diseases are primarily mediated by the elevated expression of the SASP. The SASP comprises a repertoire of factors such as TGF-β family members, VEGF, and chemokines, which contribute to the propagation of senescence to neighboring cells (Di Micco et al., 2021; Huang et al., 2022). This senescence spread, along with the interplay between the SASP and immune cells like NK cells, macrophages, and T cells, exacerbates local and systemic inflammation (Sagiv & Krizhanovsky, 2013). Additionally, the proteases and growth factors present in the SASP disrupt tissue microenvironments and facilitate cancer metastasis. Furthermore, fibrogenic and tissue remodeling factors within the SASP contribute to fibrosis in various tissues, including the skin, liver, kidney, lung, cardiac tissue, pancreas, and skeletal muscle (Schafer, Haak, Tschumperlin, & LeBrasseur, 2018).

Overall, the accumulation of senescent cells beyond a threshold and the persistence of proapoptotic, pro-inflammatory senescent cells can have significant implications for tissue health, immune function, and disease development. Understanding these processes may offer insights into potential therapeutic strategies to target senescent cells and mitigate their detrimental effects, potentially improving health outcomes in aging and age-related conditions.

Therapeutic Strategies Targeting Senescent Cells

The accumulation of senescent cells in tissues, accompanied by the detrimental effects of the SASP, is a significant contributor to the process of aging and the development of age-related diseases. Consequently, targeting senescent cells has emerged as a promising therapeutic approach for extending healthspan and mitigating various chronic age-related conditions. By either removing senescent cells or suppressing the SASP, senotherapeutic interventions aimed at reducing the burden of senescent cells hold great potential for promoting healthier aging and improving overall well-being (Chaib, Tchkonia, & Kirkland, 2022).

Several senotherapeutic strategies targeting senescent cells are being explored, including conventional small-molecule drugs, nanomedicine, and immunotherapy. Each approach offers unique advantages and challenges in selectively eliminating or modifying senescent cells for therapeutic purposes. By leveraging these diverse strategies, researchers aim to develop effective interventions that specifically target senescent cells while minimizing off-target effects. Given the promising benefits observed through the elimination of senescent cells, researchers in both academic and industry sectors are actively exploring novel approaches and agents that can effectively remove senescent cells or mitigate their harmful effects. These therapeutic strategies can be categorized into two main groups: senolytics, which focus on eliminating senescent cells, and senomorphics, which aim to counteract the detrimental effects of senescent cells by suppressing the SASP.

Senolytics

In order to avoid self-destruction by their own proapoptotic/tissue-destructive SASP, senescent cells frequently upregulate one or more senescent cell antiapoptotic pathways (SCAPs) including the BCL-2 family proteins, which can serve as molecular targets for the elimination of senescent cells (senolysis). The first senolytic agents selected by bioinformatic analyses were compounds that: (i) target multiple SCAPs, deviating from the conventional drug development approach that targets one molecular pathway with one drug for one specific disease, (ii) are either natural products with established safety profiles or already approved in the clinic for other medical purposes. Notably, among the compounds meeting these criteria are dasatinib, a well-established SRC/tyrosine kinase inhibitor with a favorable safety profile, as well as the natural flavonoids quercetin and fisetin, which are naturally present in various fruits and foods (Chaib et al., 2022).

The proof-of-concept for targeting senescent cells was first demonstrated by the senolytic combination of dasatinib and quercetin (D+Q) (Zhu et al., 2015), paving the way for the development of other senolytic agents. Among the reported senolytics are inhibitors of antiapoptotic BCL-2 family proteins, as well as HSP90 inhibitors, USP7 inhibitors, p53 modulators, and several others (Zhang et al., 2023). To date, the two most studied senolytics are D+Q and fisetin, both of which have entered different clinical trials for treatment of age-related diseases. Importantly, the administration of dasatinib and quercetin has shown effectiveness in clinical trials among patients with diabetic kidney disease, Alzheimer's disease, and idiopathic pulmonary disease (Zhang et al., 2022).

A novel approach to induce senolysis capitalizes on the characteristic enzymatic activity of senescent cells, i.e., the increased lysosomal senescence-associated β-galactosidase (SA-β-gal). By leveraging this enzymatic activity, it is possible to design galactose-capped prodrugs that exploit the action of SA-β-gal that hydrolyzes the β-glycosidic bond formed between a galactose and its organic moiety. These prodrugs involve covalently attaching galactose or acetyl galactose groups to cytotoxic drugs. Promising results have been obtained using various galactose-based prodrugs, where the cytotoxic agents employed include chemotherapeutic reagents like gemcitabine, duocarmycin, and 5-fluorouracil, among others (Cai et al., 2020; Gonzalez-Gualda et al., 2020; Guerrero et al., 2020). This prodrug strategy enhances the selective elimination of senescent cells while minimizing the impact on non-senescent normal cells. This selectivity arises from the preferential processing of the galactoside prodrugs in senescent cells following cellular uptake, leading to the targeted release of cytotoxic drugs specifically within senescent cells.

Senomorphics

An alternative approach to complete elimination of senescent cells through senolysis is the use of senomorphic agents, also known as senostatics. Senomorphics aim to disrupt key features of senescence, particularly the production and secretion of the SASP, while keeping the cells alive. By inhibiting the SASP, senomorphics have the potential to interfere with the proinflammatory nature of senescent cells and potentially delay aspects of aging and age-related diseases. This approach may also allow the immune system to play a role in reducing the burden of senescent cells by preventing the spread of senescence.

Most senomorphics exert their effects by targeting transcriptional regulators involved in SASP regulation, such as ATM, p38 MAPK, JAK/STAT, NF-κB, and mTOR pathways (Zhang et al., 2023). Studies have demonstrated that blocking the NF-κB-dependent SASP can delay the onset of progeroid symptoms and extend lifespan in mouse models of accelerated aging (Tilstra et al., 2012). Additionally, modulating the SASP through inhibition of mTOR signaling, such as with rapamycin, significantly impairs the SASP, reduces inflammation, and extends healthspan. Inhibition of mTOR can ameliorate cellular senescence by suppressing peroxisome proliferator-activated receptor-γ coactivator 1β-dependent mitochondrial biogenesis, reducing reactive oxygen species (ROS) production, and attenuating persistent activation of the DDR (Correia-Melo et al., 2016).

One potential limitation of senomorphics is the requirement for continuous treatment to maintain suppression of the SASP, in contrast to senolytics that only require intermittent administration. Continuous administration of senomorphics may increase the risk of side effects compared to intermittently dosed senolytics. It may also lead to off-target effects due to the suppression of cytokine secretion, even when such cytokines are necessary, by non-senescent cells such as innate or adaptive immune cells. Furthermore, some SASP inhibitors may have agent-specific off-target effects. For example, rapamycin has been associated with nephrotoxicity, metabolic impairment, and increased susceptibility to infections (Neff et al., 2013). While senomorphics offer an alternative approach to senolysis, further research is needed to better understand their efficacy, long-term effects, and potential side effects. Balancing the suppression of the detrimental aspects of senescence with the preservation of beneficial immune functions remains a challenge in the development of senomorphic agents for therapeutic purposes.

Immunotherapy

Organisms possess an inherent senolytic system referred to as immunosurveillance, which plays a crucial role in the elimination of senescent cells. In normal physiological conditions, senescent cells can activate both innate and adaptive immune responses by releasing SASP factors or upregulating specific surface antigens. These mechanisms facilitate the recruitment of immune cells, including T cells, macrophages, NK cells, and neutrophils, to eliminate senescent cells (Burton & Stolzing, 2018; Prata, Ovsyannikova, Tchkonia, & Kirkland, 2018). However, the aging process also affects the immune system, leading to a decline in immunosurveillance, a phenomenon known as immunosenescence. Additionally, senescent cells may develop mechanisms of immune suppression that enable them to evade immune clearance. Consequently, immunotherapy approaches aimed at enhancing the ability of immune cells to target senescent cells offer an alternative strategy for senotherapy. By stimulating the immune response against senescent cells, it is possible to bolster the clearance of these cells and potentially mitigate their detrimental effects.

Chimeric antigen receptor (CAR) T cell therapy is an anticancer approach that involves genetically modifying T cells to express an artificial receptor, enabling them to recognize and eliminate targeted cancer cells. In a recent study, CAR T cells were engineered to target urokinase-type plasminogen activator receptor (uPAR) present on the surface of senescent cells, demonstrating their potential to selectively eliminate senescent cells (Amor et al., 2020). Another immunotherapeutic approach called antibody-dependent cellular cytotoxicity (ADCC) exploits the use of antibodies to direct immune cells in clearing target cells through cytotoxic mechanisms. A novel ADCC strategy targeting dipeptidyl peptidase 4 (DPP4) was developed, wherein DPP4-expressing senescent cells were labeled with an anti-DPP4 antibody to guide NK cells for selective elimination of DPP4-positive senescent cells (Kim et al., 2017). Senolytic vaccination represents an alternative approach for senolytic

immunotherapy and offers the advantage of requiring only a few treatments or even a single administration. In one example, a senolytic vaccine targeting glycoprotein non-metastatic melanoma protein B (GPNMB) was developed using GPNMB-derived peptides. The vaccination resulted in a reduction of GPNMB-positive senescent cells, improvement in age-related pathological features, and an extension of lifespan (Suda et al., 2021).

Nanomedicine

The contribution of nanomedicine in drug delivery involves designing and utilizing nanoparticle carriers to enhance drug targeting, improve drug stability, and control drug release for more effective and precise treatment. Various nanoparticles have been designed as nanocarriers to target senescent cells for therapeutic interventions (Morsli, Doherty, & Munoz-Espin, 2022). For example, selective delivery of cytotoxic drugs like doxorubicin and navitoclax to senescent cells can be achieved by conjugating drug-loaded nanoparticles with galacto-oligosaccharides that are recognized and hydrolyzed by SA-β-gal for free drug release to selectively kill senescent cells while sparing normal healthy cells (Munoz-Espin et al., 2018). Additionally, targeting senescence-specific surface proteins such as CD9 or β2-microglobulin (B2M) using monoclonal antibody-conjugated nanoparticles or molecularly imprinted nanopolymers (nanoMIPs) allows for enhanced uptake of senolytic drugs like rapamycin or dasatinib, leading to specific killing of senescent cells while minimizing off-target toxicity (Ekpenyong-Akiba et al., 2019).

Conclusion and Perspectives

The accumulation of senescent cells and their associated proinflammatory/tissue-destructing SASP, is a significant driver to aging and age-related diseases. Targeting senescent cells through senolysis or modulating their effects through senomorphics represents promising therapeutic strategies for extending healthspan and delaying age-related conditions. Despite the progress made in understanding and developing senotherapeutic approaches, there are several avenues for future exploration. Further research is needed to identify more efficient and specific senolytic agents with minimal off-target effects. Understanding the complex signaling pathways and transcriptional regulators of the SASP will aid in the development of potent senomorphics that selectively modulate the SASP without impairing necessary immune functions. Additionally, the development of innovative delivery systems, such as targeted nanocarriers, can enhance the efficiency and specificity of senotherapeutic interventions.

Furthermore, the long-term effects and potential side effects of senotherapeutic approaches require careful evaluation. Balancing the suppression of senescence-related pathology with the preservation of essential cellular functions and immune responses remains a challenge. Studies investigating the impact of senolytics and senomorphics on overall health, lifespan, and age-related diseases in preclinical models and clinical trials will provide valuable insights into their therapeutic potential and safety. In conclusion, targeting senescent cells and modulating their effects through senolysis and senomorphics offer exciting prospects for mitigating the detrimental effects of aging and age-related diseases. Continued research and development in this field have the potential to revolutionize the way we approach aging and improve healthspan in the future.

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