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Zombie cells in Ageing: A comprehensive narrative review on Mechanisms and Opportunities

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Abstract

As the human body ages, the prevalence of age-related diseases and declination of life span have become significant global issues. Among the many hallmarks of ageing, cellular senescence plays an important role, with senescent cells, often called "zombie cells," accumulating in various tissues and contributing to age-related pathologies and diseases. Cellular senescence is a complex process that involves tissue remodeling and embryogenesis. However, prolonged senescence leads to malignancies and age-related diseases. Cellular senescent cells exhibit histopathological changes like flattened cell shape, granular and vocalizational cytoplasm, and abnormal cell organelles. Therapies targeting senescent cells include senolytic and senomorphic drugs, stem cells, and extra vesicular management, improving life span and reducing inflammation.

This narrative review aims to comprehensively explore the molecular biology of zombie cells, their role in ageing, mechanisms of senescence induction and maintenance, and their implications for age-related diseases. Furthermore, this review has studied recent advancements in therapeutic strategies targeting senescent cells and discusses future directions for research in this expanding field. By synthesizing current knowledge and highlighting gaps in understanding, this review has deepened our understanding of the complex interplay between senescent cells and ageing, identifying novel therapeutic interventions to promote healthy ageing and enhance the quality of life in an ageing population.

Keywords: Zombie cells, cellular senescent, histological changes, Senomorphic drugs, Ageing.

Introduction

Cellular senescence and "zombie cells" are intriguing molecular biological concepts, particularly in ageing and age-related diseases [1, 2]. Cellular senescence is when cells cease to divide and undergo histological changes, leading to their functional limitations [3, 4]. This process is often described as a cellular response to DNA damage, oxidative stress, or oncogenic mutations [3, 4]. Senescent cells are characterized by a range of phenotypic changes, including altered gene expression patterns, enlarged and flattened cell shape, increased secretion of pro-inflammatory molecules (a phenomenon known as the senescence-associated secretory phenotype or SASP), and resistance to apoptosis (programmed cell death) [5-7]. Even though cellular senescence plays a crucial role in processes such as embryonic development, wound healing, and tumor suppression by halting the proliferation of damaged cells, it also contributes to ageing and age-related diseases. Accumulation of senescent cells in tissues over time has been implicated in various age-related pathologies, including cancer, cardiovascular diseases, neurodegenerative disorders, and tissue fibrosis [8]. This has led to the hypothesis that targeting senescent cells could be a promising strategy for extending health span and delaying age-related diseases. "Zombie cells" refers to senescent cells that evade the body's mechanisms for clearing damaged or dysfunctional cells. Unlike healthy cells, which undergo programmed cell death (apoptosis) or are removed by the immune system when they become senescent, zombie cells linger in tissues and continue to exert harmful effects [9]. These cells are often called "zombie-like" because they are essentially dead but remain metabolically active and can release inflammatory molecules, contributing to tissue dysfunction and chronic inflammation [1, 4]. Zombie cells significantly challenge the body's ability to maintain tissue homeostasis and repair. Their accumulation is believed to exacerbate age-related decline and contribute to the development of various diseases. Therefore, strategies aimed at selectively eliminating or targeting zombie cells, such as senolytic therapies, have acquired a significant interest in ageing research [10, 11].

Studies suggest that cellular senescence and zombie cells are interconnected phenomena with profound implications for ageing and age-related diseases [6-8]. Understanding the mechanisms underlying their formation and persistence is crucial for developing interventions to promote healthy ageing and mitigate the burden of age-related disorders. Hence, the main aim of this review is to understand the histological overview, immunohistochemical assessments, role, and molecular pathway of zombie cells in age-related diseases, along with challenges and

opportunities. Furthermore, this review will examine recent advancements in therapeutic strategies targeting senescent cells and discuss future directions for research in the related field. By synthesizing current knowledge and highlighting gaps in understanding, this review will deepen the understanding of the complex interplay between senescent cells and ageing to identify novel therapeutic interventions to promote healthy ageing and enhance the quality of life in an ageing population.

Historical overview of Zombie cells

In recent years, cellular senescence, particularly "zombie cells," has gained the interest of medical professionals. Hayflick and Moorhead 1960 observed that few cells had limited capacity to divide and related those cells with ageing [12]. Further research revealed that these cells are hallmarks of ageing, which can alter the lysosomal activity, increase the expression of anti-proliferative protein, alter the cell size and morphology, and show resistance to apoptosis.

Another important characteristic recorded was regulation in secretion of interleukin, growth, and inflammatory factors; because of this activity of cells, they are further recognized as senescence-associated secretory phenotypes (SASP) [13, 14].

Dr James Kirkland and colleagues 2011 coined the term "zombie cells" to describe these persistent, metabolically active but functionally compromised senescent cells that accumulate with age [15-17]. They identified these cells as contributors to age-related tissue dysfunction and coined the term "senolytics" for therapies to eliminate these zombie cells selectively. In summary, while the understanding of cellular senescence dates back several decades, the recognition of persistent, functionally compromised senescent cells as "zombie cells" is a more recent development in the field of ageing research [18]. The concept underscores the importance of addressing cellular senescence and its consequences for healthy ageing and disease prevention.

Molecular Mechanisms Underlying Senescence Induction and Maintenance

In the studies present in the literature, cellular activities like telomere attrition, oncogenic mutations, and DNA damage induce the stress response mechanism [10, 19]. Figure 1 explains the inducers of zombie cells. Cellular senescence, a state of irreversible growth arrest, can be triggered by various intrinsic and extrinsic factors[19, 20]. DNA damage from sources like UV radiation, ionizing radiation, chemicals, or replication errors can initiate senescence, as can oxidative stress caused by reactive oxygen species (ROS) either from normal cellular metabolism

or environmental exposure[21]. Telomere shortening, a natural consequence of cell division, leads to senescence when telomeres become critically short, preventing genomic instability and cancer. [22] Genetic factors, including mutations in genes governing cell cycle regulation, DNA repair, or senescence pathways, can predispose cells to premature senescence[23]. Oncogene activation, promoting cell proliferation and survival, can induce senescence as a tumor-suppressive mechanism[24, 25]. Various stress signals from the cellular microenvironment, such as inflammation or growth factors, can also trigger senescence. Mitochondrial dysfunction, epigenetic changes , metabolic dysregulation, and the senescence-associated secretory phenotype (SASP), involving the secretion of factors like pro-inflammatory cytokines, growth factors, and proteases, collectively contribute to cellular senescence and tissue dysfunction [26-28].

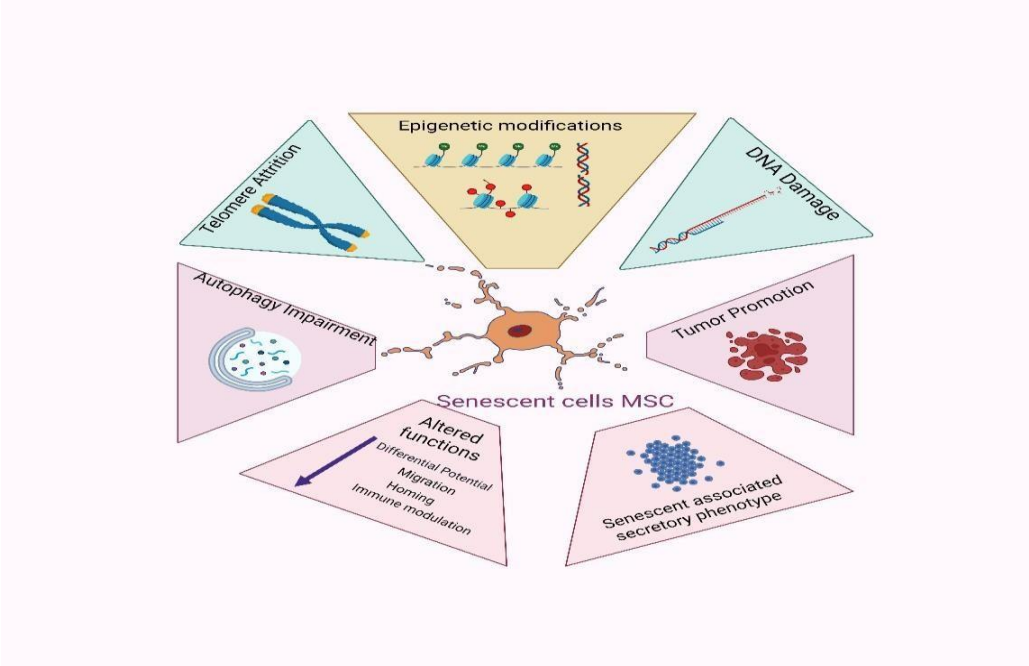

Figure 1: Inducers of Zombie cells

Difference between normal cells and zombie cells: Morphological and metabolic changes

Table 1 and Figure 2 demonstrate the difference between normal cells and zombie cells. Normal cells and zombie cells (senescent cells) exhibit distinct morphological and metabolic changes, reflecting their different bodily states and functions [29, 30].

Table 1: Morphological and metabolic differences between zombie cells and normal cells

Morphological difference	
Zombie cells	**Normal cells**
Enlarged and flattened cells with irregularly shaped boundaries	Regular and uniform shaped with defined boundaries
Develops vacuoles and possesses irregular cytoplasmic granularity	Defined organelle and structures
Formation of senescence-associated heterochromatin foci (SAHFs)and alteration in chromatin structure.	High nucleus-to-cytoplasm ratio
Resistant to apoptosis but metabolically active	Undergoes regular mitosis as a part of normal physiological process
Metabolic difference	
Increased metabolic activity, including elevated glycolysis (the Warburg effect), and decreased mitochondrial respiration.	Balanced and regulated metabolism to support cellular functions, growth, and proliferation.
Accumulate lipids, glycogen, and other metabolic byproducts due to impaired clearance mechanisms.	Utilize nutrients for energy production, biosynthesis, and maintenance of cellular homeostasis.
Cells can secrete pro-inflammatory cytokines, growth factors, and other signalling molecules as part of the senescence-associate phenotype (SASP).	Respond dynamically to extracellular signals and adjust their metabolic activity to meet cellular demands.

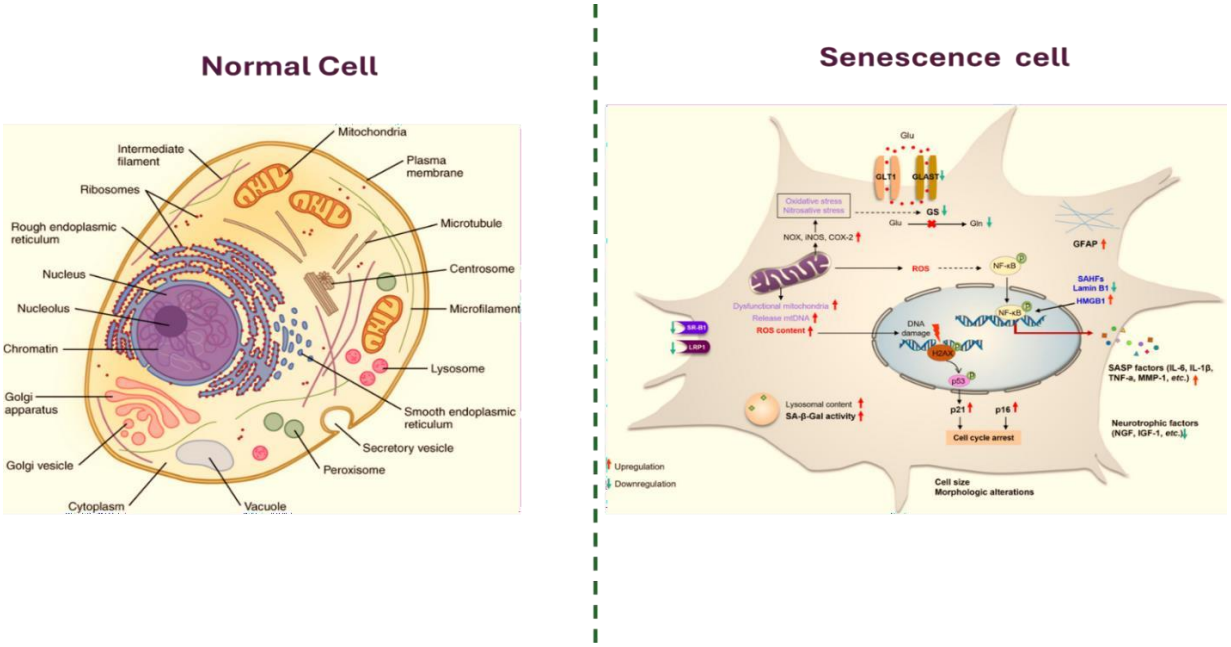

Figure 2: Difference between normal cells and Zombie cells

The induction and maintenance of cellular senescence involves a complex interplay of molecular mechanisms, including signalling pathways, epigenetic regulation, and changes in gene expression (Figure 3).

DNA Damage Response (DDR) Pathway: Cellular senescence can be triggered by DNA damage, including double-strand breaks, telomere attrition, and oxidative damage. Activating DDR pathways involving proteins such as ATM, ATR, CHK1, and CHK2 leads to cell cycle arrest and senescence induction [31, 32].

Senescence-Associated Secretory Phenotype (SASP): Senescent cells secrete various factors collectively known as SASP, including pro-inflammatory cytokines, chemokines, growth factors, and proteases [33, 34]. SASP components contribute to inflammation, tissue remodelling, and the propagation of senescence to neighbouring cells through paracrine signalling.

p53-p21 and p16-RB Pathways: Two major tumor suppressor pathways, mediated by the proteins p53 and p16INK4a, play crucial roles in regulating cellular senescence [35-38]. Activation of p53 leads to the upregulation of p21, a cyclin-dependent kinase inhibitor that induces cell cycle arrest. Similarly, the p16INK4a protein inhibits CDK4/6-mediated phosphorylation of RB, resulting in cell cycle arrest and senescence [35-38].

Telomere Dysfunction: Progressive telomere shortening with cell division eventually triggers a DNA damage response, leading to replicative senescence [39-41]. Telomere dysfunction can also be induced prematurely by various stresses, such as oxidative stress or oncogene activation, contributing to senescence induction [39-41].

Epigenetic Changes: Senescence is associated with extensive alterations in chromatin structure and epigenetic modifications. These changes can include global chromatin condensation, senescence-associated heterochromatin foci (SAHFs) formation, and changes in histone modifications and DNA methylation patterns, which regulate gene expression during senescence [42].

Mitochondrial Dysfunction: Dysfunctional mitochondria and increased production of reactive oxygen species (ROS) contribute to senescence induction [43]. ROS can cause oxidative damage to cellular components, activate signalling pathways in senescence, and promote the expression of SASP factors.

Inflammatory Signaling: Inflammatory pathways, such as NF-κB and the JAK/STAT pathway, are activated during senescence and contribute to the maintenance of the SASP and the pro-inflammatory microenvironment associated with senescent cells [44].

Nutrient Sensing Pathways: Senescence can be influenced by nutrient availability and metabolic signalling pathways such as mTOR and AMPK [45]. Dysregulation of nutrient-sensing pathways can impact cellular metabolism and contribute to senescence induction and maintenance.

Understanding these molecular mechanisms underlying senescence induction and maintenance is crucial for developing strategies to modulate senescence-associated processes and potentially mitigate age-related diseases and tissue dysfunction.

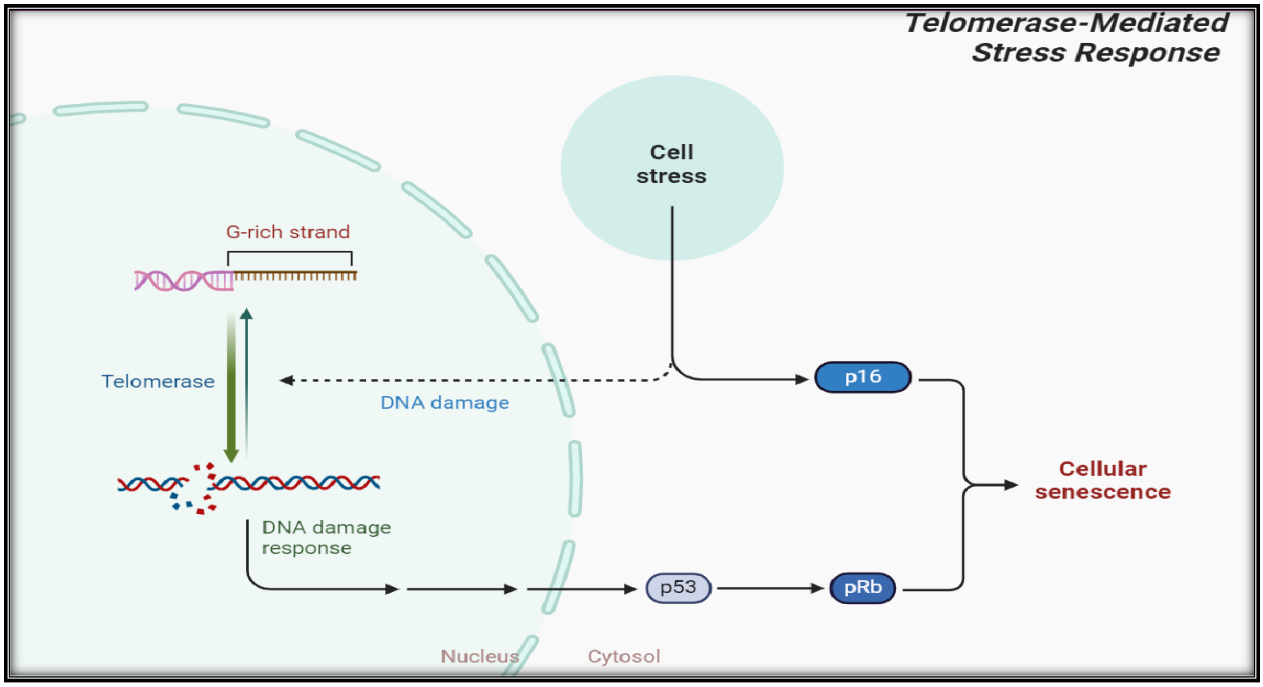

Figure 3: Molecular Mechanism underlying cellular senescence

Role of Zombie Cells in Age-Related Diseases: From Cancer to Neurodegeneration

Age-related diseases pose significant challenges to global healthcare systems, contributing to increased morbidity and mortality among ageing populations. Among the various factors implicated in these diseases, the emergence of "zombie cells," or senescent cells, has drawn considerable attention. Senescent cells, once considered a protective mechanism against cancer,

are now recognized as key contributors to age-related pathologies spanning from cancer to neurodegeneration.

Inflammaging

The ageing process can be characterized by continuous progression and reduction in the biological functionality of the organism. Even though the immune system is believed to have a higher capacity for renewal, it is also affected by the ageing process as this process is known as immunosenescense [46] (figure 4). This process contributes to increasing geriatric individual susceptibility towards infection, autoimmune diseases, and tumors.

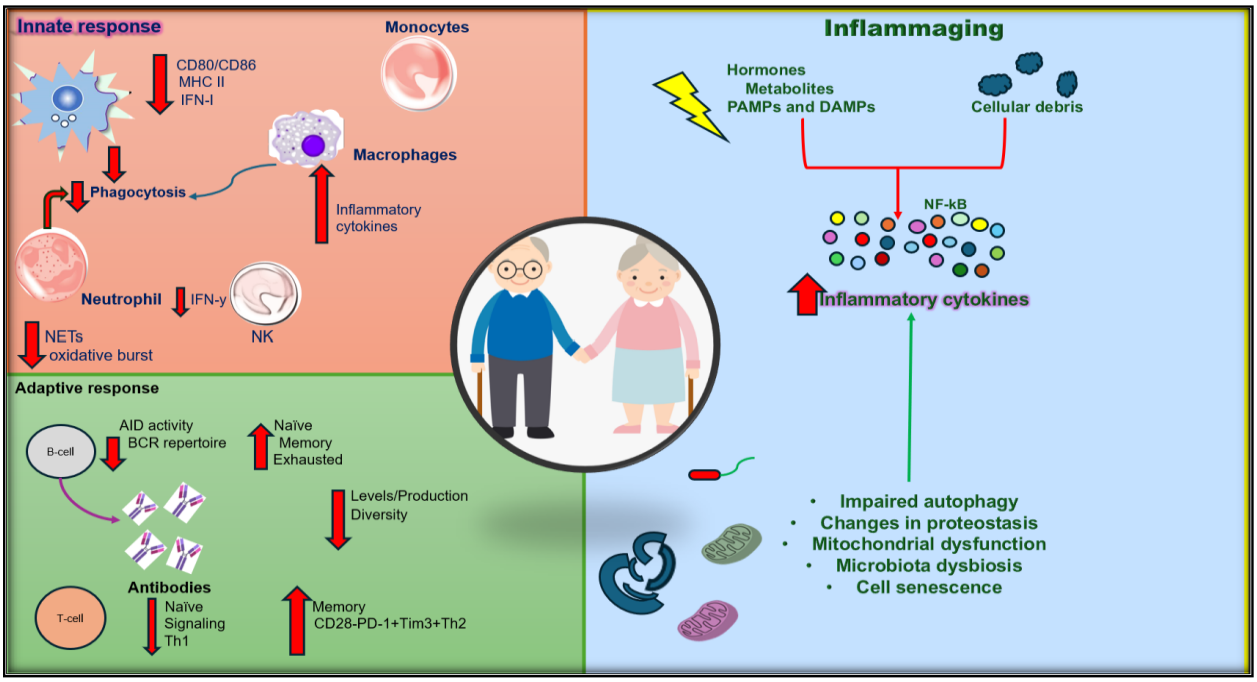

Figure 4: Major immunological alterations observed during immunosenescence.

Ageing interferes with a few innate and adaptive immune cells aspects that can impair or compromise their function and response. Additionally, several factors can dysregulate intracellular homeostasis during ageing, intensifying the secretion of inflammatory cytokines and chemokines (Inflammaging).

The most striking feature of the immunosenescence process is a low-grade proinflammatory state, with increasing serum mediators like IL-6, TNF-α, IL-1, and c-reactive protein [47, 48].

This low-grade inflammatory stat is associated with the reduced activity of cells to mount immune response during the ageing process (figure 4).

Inflammaging arises from a complex interplay of hormonal, metabolic, and immune factors that continually stimulate innate receptors, fostering a pro-inflammatory reaction within the body [15, 49]. Additionally, senescent cells undergo intracellular changes, including telomeric alterations and oxidative stress, leading to the activation of signalling pathways such as nuclear factor κB (NF-κB) [16, 17, 47]. This activation results in increased secretion of cytokines, chemokines, growth factors, and lipids, collectively termed the senescence-associated secretory phenotype (SASP), which can contribute to inflammaging. Furthermore, the heightened inflammatory response observed with ageing may stem from a diminished capacity to resolve inflammation, as several regulatory factors are deficient in older individuals [46].

Senescence and Cellular Dysfunction:

Cellular senescence is a state of irreversible growth arrest characterized by alterations in cell morphology, gene expression, and secretory phenotype. Initially identified as a tumor suppressor mechanism to prevent the proliferation of damaged cells, senescence has since been implicated in the pathogenesis of various age-related diseases [50]. Senescent cells accumulate in tissues over time due to DNA damage, telomere attrition, oxidative stress, and chronic inflammation.

Role in Cancer:

In cancer, senescent cells exhibit a Janus-faced behavior, initially suppressing tumorigenesis by halting the proliferation of damaged cells. However, the persistent presence of senescent cells in the tumour microenvironment contributes to tumour progression through the senescence-associated secretory phenotype (SASP) [51]. The SASP promotes inflammation, tissue remodelling, angiogenesis, and immune evasion, fostering a pro-tumorigenic environment that fuels tumour growth, invasion, and metastasis [50]. Targeting senescent cells or modulating the SASP holds promise as a therapeutic strategy to augment conventional cancer treatments and prevent tumour recurrence.

Contribution to Cardiovascular Disease:

Senescent cells play a detrimental role in age-related cardiovascular diseases, including atherosclerosis, hypertension, and heart failure. In vasculature, senescent endothelial cells and vascular smooth muscle cells impair vascular function, promote inflammation, and contribute to plaque formation and arterial stiffness [52, 53]. The SASP exacerbates vascular dysfunction by promoting endothelial dysfunction, oxidative stress, and smooth muscle cell proliferation.

Strategies aimed at eliminating senescent cells or attenuating the SASP offer potential

therapeutic avenues to mitigate age-related cardiovascular dysfunction and reduce the burden of cardiovascular disease [52, 53].

Implications in Neurodegenerative Diseases:

Senescent cells have an emerging role in age-related neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS). In the central nervous system, senescent glial cells, including astrocytes and microglia, drive neuroinflammation, synaptic dysfunction, and neuronal damage [20]. The SASP contributes to the propagation of neurodegenerative processes by promoting the accumulation of misfolded proteins, oxidative stress, and neuronal apoptosis. Targeting senescent cells in the brain represents a promising therapeutic approach to attenuate neuroinflammation, preserve neuronal function, and delay the onset or progression of neurodegenerative diseases [54, 55].

Senescent cells, once regarded as guardians against cancer, have emerged as prominent culprits in age-related diseases, encompassing cancer, cardiovascular disease, and neurodegeneration. The senescence-associated secretory phenotype fuels chronic inflammation, tissue dysfunction, and disease progression, underscoring the importance of targeting senescent cells or modulating their secretory phenotype in therapeutic interventions. Strategies to eliminate senescent cells or reduce the SASP offer promising avenues for mitigating age-related diseases and promoting healthy ageing [56]. Continued research into the role of senescent cells in age-related pathologies is essential for developing effective therapies to address the growing burden of age-related diseases in ageing populations.

Assessment of zombie cells by immunohistochemical (IHC) and ultrastructural methods

Table 2 outlines the hallmarks of cellular senescence and presents the informative-rich techniques employed for detecting these cells in vitro. Below, a detailed explanation of IHC and ultrastructural methods is given.

Immunohistochemistry (IHC):

Immunohistochemistry involves using antibodies to detect specific proteins within tissue sections. Researchers typically target senescent cell markers associated with senescence, such as p16^INK4a, p21^Cip1/Waf1, senescence-associated β-galactosidase (SA-β-gal), and others [57-60]. Antibodies against these markers are applied to tissue sections, and if the target proteins are present in the cells, they will bind to the antibodies. Visualization of the bound antibodies is

usually achieved through colourimetric or fluorescent techniques, allowing researchers to identify and quantify senescent cells within the tissue [57-60].

Ultrastructural Methods:

Ultrastructural methods, such as transmission electron microscopy (TEM), provide high-resolution images of cellular structures. Senescent cells may exhibit characteristic ultrastructural features, such as enlarged and flattened morphology, increased lysosomal activity, altered chromatin organization, and the presence of cytoplasmic vacuoles [61-63]. TEM allows researchers to visualize these features at the ultrastructural level, providing insights into the cellular changes associated with senescence.

Researchers can comprehensively assess senescent cells within tissues by combining immunohistochemical staining with ultrastructural analysis [64]. Immunohistochemistry provides specific identification of senescent cells based on protein markers, while ultrastructural methods offer detailed visualization of cellular morphology and organelles, enhancing our understanding of the structural changes associated with cellular senescence. This integrated approach enables researchers to study the presence, distribution, and characteristics of senescent cells in various tissues and their potential roles in ageing and age-related diseases .

Table 2: Biomarkers and detection methods of cellular senescence (Zombie cells)

Senescent cell hallmark	Marker	Specific senescent subtype	Detection method
Cell cycle arrest			
Lack of DNA synthesis	BrdU, EdU,	General, Staining incorporation,	immunohistochemistry (IHC)
Lack of proliferation	pRB, Ki67,	General	IHC
Activation of p16-pRB axis	p16INK4a, phospho-pRb	General	IHC
Activation of p53-p21	axis p21, p53, phospho-p53, DEC1 (BHLHB2), PPP1A	General, damage-induced, oncogene-induced	IHC, Immunofluorescence (IF)
Structural changes			

Morphology, cell size	Morphology, cell size	General	Light microscopy, transmission electron microscopy
Increased lysosomal compartment and activity	SA-galactosidase SA-a-Fucosidase Lipofuscin	General	Enzymatic staining, Dye incorporation
DNA damage	cH2AX, 53BPI, Rad17, ATR, ATM, MDC1, TIF	General, damage-induced	Immunofluorescence, transmission electron microscopy
Reactive Oxygen Species (ROS)	ROS	Oxidative stress-induced, general	Chemiluminescent, fluorometry
Telomere shortening SAHFs formation	Replicative-induced qPCR, SAHFs, H3K9-Methylation, HP1-gamma	General, damage-induced	IHC, IF
Nuclear membrane	Lamin B1	General	IF, WB
Pro-survival			
Apoptosis exclusion	Annexin V, Cleaved PARP, Cleaved caspase, TUNEL staining	General	IF, IHC
SASP, Cytokine secretion	IL-6, IL-8, CXCR2, IGF2, IGFBP3, IGFBP5, IGFBP7, STC1 GDF15, SERPINs	Damage-induced, oncogene-induced senescence (OIS)	ELISA, WB, SASP-responsive alkaline phosphatase assay
Plasma membrane protein expression	ICAM-1, DEP1, B2MG, NOTCH3, DcR2	Replicative induced, General	IHC, IF, WB, flow cytometry
*WB-western blot; IF-immunofluorescence.			

Senolytic Therapies

As the literature suggests, cellular senescence plays a significant role in age-related diseases. Interventions targeting to cease the formation of these cells are known as senotherapeutic (Table 3). The experimental study on mice for kidney injury and improved life span has shown promising results with these interventions [65]. Existing senotherapeutic interventions include medications that help to slow down or kill senescent cells; these drugs are known as senolytics. The drugs that inhibit the growth of SASP are known as senomorphics, which are exogenous

cell-based drugs . Various lifestyle interventions have been proven to reduce the growth of senescent cells (Table 3).

Table 3: Senolytic therapy

Senotherapeutic approach	Type	Examples
Senolytic therapies	Apoptosis inducers	AP20187, navitoclax, EF24, venetoclax, A-1155463, antibody-engineered, ginsenoside, Quercetin, A-1331852.
	Immunotherapies	Chimeric antigen receptor, activator of invariant natural killer, vaccines
Senomorphics	senescence-associated secretory phenotype regulators	Metformin, glucocorticoids, melatonin, oestrogen resveratrol, ruxolitinib, androgen, oestradiol, and rapamycin.
Stem cells and extracellular vesicles	Stem cells	Bone marrow mesenchymal stem cells, pluripotent stem cells, umbilical cord-derived mesenchymal stem cell
	Stem cell-derived extracellular vesicles	mesenchymal stem cell-derived vesicles, dental pulp stem cell-derived vesicles, antler stem cell-derived vesicles
Non-pharmacological interventions	Lifestyle modifications	Regular exercise, diet control, restriction on calorie intake
	Others	Fractional micro-needling radiofrequency treatment, radio-electric asymmetric conveyer technology

Senolytics are compounds or treatments designed to selectively induce apoptosis (cell death) in senescent cells while sparing healthy cells. By explicitly targeting senescent cells, senolytics aim to reduce the burden of senescent cells in tissues and alleviate the pro-inflammatory and pro-tumorigenic effects associated with cellular senescence [66-69]. Senolytics exert their effects through various mechanisms, including targeting anti-apoptotic pathways upregulated in

senescent cells, such as the BCL-2 family of proteins. By inhibiting these pathways, senolytics sensitize senescent cells to apoptosis, leading to their selective elimination. Additionally, senolytics may target specific vulnerabilities or signalling pathways dysregulated in senescent cells, making them susceptible to cell death induction [66-69]. Senolytic interventions encompass a diverse range of compounds and approaches. These include small molecules, peptides, antibodies, and natural compounds demonstrating senolytic activity in preclinical and clinical studies. Examples of senolytic compounds include dasatinib, quercetin, fisetin, and navitoclax. Additionally, innovative approaches such as gene therapy and nanomedicine are being explored for their potential to target senescent cells selectively [70, 71].

Senolytic interventions hold promise for the treatment and prevention of a wide range of age-related diseases, including cancer, cardiovascular disease, neurodegenerative disorders, osteoarthritis, and pulmonary fibrosis. By reducing the burden of senescent cells in tissues, senolytics can potentially alleviate chronic inflammation, tissue dysfunction, and disease progression associated with ageing and age-related pathologies [2, 3, 54]. Despite their therapeutic potential, senolytic interventions face several challenges. These include off-target effects, potential toxicity to non-senescent cells, and optimizing dosage and delivery methods. Additionally, the heterogeneity of senescent cells within tissues and the context-dependent effects of senolytics necessitate further research to refine their efficacy and safety profiles.

Senomorphic Drugs

Metformin is a well-known senomorphics drug that helps in reducing age-related diseases and improves lifespan among patients with type-2 diabetes. Research on metformin has shown its ability to enhance the anticancer efficacy of CDK6 and CDK4 inhibitors by tempering with SASP [5, 13, 55, 58]. These cells inhibit endothelial senescence formed by glucose-induced metabolic memory and regulate the sirtuin pathway by triggering immune-medicated senescent cells. Other senomorphic drugs include JKA inhibitors (rixolitinib) that reduce inflammation and improve the life span, as reported in studies on mice [7]. Rapamycin, an mTOR inhibitor, helps suppress SASP in endothelial cells and reduces organ damage.

Senomorphic drugs also include herbal extracts, like resveratrol, that slow down SASP activity. Furthermore, this herbal drug helps reduce or eliminate tumor-senescence cells. However, studies suggest that these drugs should be used with caution to avoid side effects that sometimes lead to incongruent behaviour of tumours [5, 11, 61, 62]. Hormones such as melatonin also have

senomorphic effects. Studies have supported that these hormones suppress SASP gene expression by interrupting CREB-protein binding cycles, leading to senor DNA damage. Other hormones that have anti-SASP properties are androgens, estrogens, estradiol, and glucocorticoids [29, 30, 34]. Research suggests using glucocorticoids cautiously as they may induce senescence in primary human tenocytes [43, 48, 52].

Stem cells and Extracellular vesicles

Using stem cells and extracellular vesicles (EVs) in senolytic treatment represents an innovative approach to targeting senescent cells and promoting tissue regeneration and repair. With their regenerative and immunomodulatory properties, stem cells have shown promise in rejuvenating ageing tissues, while EVs, nanosized membrane-bound vesicles secreted by various cell types, including stem cells, possess potent paracrine signalling capabilities [43, 45, 49, 70, 71]. Stem cells, including mesenchymal stem cells (MSCs), can migrate to sites of injury or inflammation and modulate the local microenvironment through paracrine signalling and direct cell-to-cell interactions. Studies have demonstrated that stem cells exhibit senolytic effects by selectively targeting senescent cells for clearance through direct cell-cell contact or via secreted factors [1, 5, 6]. MSCs have been shown to secrete factors that induce apoptosis in senescent cells, such as tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) and soluble Fas ligand (sFasL), while sparing healthy cells [9, 13, 18]. Preclinical studies using stem cell-based therapies have shown promising results in reducing the burden of senescent cells and ameliorating age-related pathologies in various tissues, including the cardiovascular, musculoskeletal, and central nervous systems [14, 23].

Extracellular vesicles (EVs), including exosomes and microvesicles, are released by stem cells and other cell types and contain a cargo of proteins, lipids, nucleic acids, and other bioactive molecules. EVs derived from stem cells possess senolytic properties and can selectively target senescent cells by transferring bioactive molecules, including microRNAs, proteins, and lipids. Studies have shown that EVs derived from MSCs can induce apoptosis in senescent cells, reduce the senescent cell burden, and alleviate age-related tissue dysfunction in preclinical models [24, 31, 61]. EVs offer several advantages as senolytic agents, including their ability to traverse biological barriers, low immunogenicity, and potential for targeted delivery to specific tissues or cell types [25, 63].

Combining stem cell therapy with EV-based approaches holds promise for enhancing the efficacy of senolytic treatment. Stem cells can act as a source of EVs, releasing senolytic EVs into the local microenvironment to target senescent cells. Additionally, stem cells can provide a supportive niche for EVs, enhancing their stability, bioavailability, and therapeutic potency.

Preclinical studies investigating combination therapies involving stem cells and EVs are underway to evaluate their synergistic effects on senescent cell clearance and tissue regeneration in age-related diseases [28, 72].

Lifestyle modifications

Lifestyle interventions are an integrated approach to senolytic treatment by modulating cellular senescence, reducing the burden of senescent cells, and promoting healthy ageing. While pharmacological senolytic agents target senescent cells directly, lifestyle interventions address underlying factors contributing to cellular senescence and promote overall health and well-being [30]. Caloric restriction, without malnutrition, has been shown to delay ageing and reduce the accumulation of senescent cells in various tissues. It involves reducing calorie intake while maintaining adequate nutrition, which may activate cellular pathways involved in longevity and promote the clearance of senescent cells. Diets rich in antioxidants, such as fruits, vegetables, nuts, and seeds, can help mitigate oxidative stress, a key driver of cellular senescence.

Antioxidants scavenge free radicals and reduce DNA damage, potentially reducing the burden of senescent cells [32, 72].

Physical activity has been associated with reduced cellular senescence and improved overall health. Exercise promotes tissue regeneration, reduces inflammation, and enhances immune function, all of which contribute to the clearance of senescent cells. Both aerobic and resistance exercises have been shown to have senolytic effects. High-intensity interval training (HIIT), characterized by short bursts of intense exercise followed by brief recovery periods, has emerged as a particularly effective form of exercise for promoting cellular rejuvenation and reducing the burden of senescent cells. The NRF2-KEAP1 signaling pathway is activated in response to cumulative lifetime exposure to environmental stressors, such as temperature, oxygen levels, and nutritional deficiencies [64, 65]. This activation triggers adaptive homeostatic mechanisms involving antioxidant and anti-inflammatory responses. Altering these exposures could potentially provide novel approaches to enhance the duration of good health and fight against chronic kidney disease (CKD), in addition to benefiting the process of cellular senescence.

Challenges and Future Directions in Senescence Research

Apart from various research and advancements made to explain the importance and utilization of cellular senescence, multiple questions are still arising in this field. For instance, the induction of senescence results in heterogeneity, with cells being in different stages of involvement of this phenotype. The presence of several distinct yet uncharacterized biomarkers and their ability to be activated poses significant challenges in identifying and targeting cells pharmacologically with senolytics and senomorphics. Furthermore, existing data suggests that senescence is a constant process, even though it's not an irreversible cellular condition. This new trait of senescent cells is attributed to the adaptability of their SASP in various situations like malignancy, reprogramming, and rejuvenation [34]. Recent research demonstrates that the SASP is not homogeneous and static as initially thought; instead, it is dynamic, modifying gradually depending on triggers, context, and cellular type [42, 43].

Additionally, every cell type has a specific pathophysiology in varied disease contexts, making it more difficult for researchers to identify a particular marker. Hence, making a universal marker to detect specific senescence cells is challenging. However, researchers suggest that combining various serotherapeutic drugs targeting SnCs cells can help improve the efficacy of a drug and will help in reducing side effects. The advantages and disadvantages of these variable SASP elements on their surroundings, specifically their relationship with the immune system, are currently being meticulously studied.

The role of cellular senescence in neurodegeneration is well-established. The cellular senescence phenotype has been found in the post-mitotic stage of neurons and oligodendrocytes, suggesting that these cells may also become senescent. Resident neuron cells are either slow-growing or post-mitotic and exhibit stress-induced premature senescence cells due to stress-inducing hormones. However, the connection between the process of neurodegeneration and the putative role of cellular senescence is still unknown and requires further investigation.

Conclusion

In conclusion, zombie cells are present in various ageing-related diseases, and their potential role in this disease has been well-documented. Altogether, the relation of these cells with curing age-related diseases has given hope to medical professionals for curing these diseases. However, developing a gold standard to identify this senescence is complex because different cells undergo different senescence depending upon their microenvironment. Moreover, with these changes,

treatment of age-related diseases becomes challenging. Furthermore, it should be emphasized that the development of senescence can be advantageous in the circumstances and for multiple medical conditions. Therefore, further investigation is necessary to comprehensively understand this complex cellular trait and its impact on the microenvironment, tissue, and organism.

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