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Guardians of Time: P16INK4A's Role in Age-Related Cellular Senescence

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Abstract:

The p16INK4a regulator is a reliable biomarker for aging that is suppressed during early embryogenesis and gradually induced throughout the aging process. Its involvement in determining cell fate contributes to aging, and its expression pathways align with those of early development, suggesting that the decline of developmental pathways may be the primary driver of aging, with damage serving as a secondary factor. Aging results in decreased functionality and increased vulnerability to death, which is the main risk factor for major human diseases. This review explores the multifaceted function of P16INK4A, shedding light on the intricate molecular mechanisms that underlie its impact on cellular senescence and its broad implications for age-related diseases. The complex interaction between P16INK4A and the senescence-associated secretory phenotype (SASP) is also discussed, revealing its effects on tissue homeostasis. The article presents a compelling perspective on the therapeutic potential of interventions targeting P16INK4A, which not only hold the promise of slowing down the aging process but also offer the opportunity to mitigate age-related diseases. Through its examination of P16INK4A's role, this article presents a captivating narrative that highlights the remarkable ability of science to unravel the mysteries of aging and potentially reshape our understanding of the guardians of time.

Keywords: p16INK4a, Cellular senescence, Pathophysiology, Treatments, WNT/b-catenin pathway

p16INK4a. an indicator of the ageing process.

The aging process is an intricate journey characterized by the gradual buildup of damage to large molecules and a decline in tissue renewal. Interestingly, this decline in tissue regenerative capacity is not necessarily linked to a decrease in the number of stem cells or their innate ability to self-renew [1]. Epidermal stem cells in both young and old mice display similar numbers and gene expression patterns [2,3,4], yet the epidermis can still maintain its essential barrier properties throughout an organism's lifespan, albeit at a slower pace. An intriguing observation is that epidermal grafts from older individuals can provide permanent coverage for burnt skin [5,6], hinting at latent regenerative potential even in aging skin.

Experiments on aged mice shed light on the factors contributing to the slower regeneration rate. This reduction in regenerative speed appears to be associated with changes in transit-amplifying (TA) cell kinetics following the accumulation of damage. This leads to a decrease in the cellular output of TA cells and a slowdown in TA cell proliferation. Surprisingly, the number of TA cells increases in aged epidermis, possibly because they progress more slowly through the cell cycle compared to their younger counterparts [3,7].

One of the key hallmarks of aging is cellular senescence, a state induced by various intrinsic and extrinsic factors, resulting in growth arrest and significant phenotypic alterations, including changes in chromatin structure and secretome. In younger organisms, cellular senescence serves as a protective mechanism, preventing the uncontrolled proliferation of damaged cells. However, in older organisms, the continuous accumulation of damage and the inadequate clearance of senescent cells lead to their persistence, with detrimental effects on tissue homeostasis [8,9,10].

The aging process is not just a biological curiosity; it has profound implications for health and disease. Aging is characterized by a progressive decline in physiological function, resulting in decreased physical fitness, heightened vulnerability to diseases, and increased mortality rates. Common age-related pathologies encompass cancer, diabetes, cardiovascular disorders, immune decline, and neurodegenerative diseases. These age-related phenotypes are closely tied to cellular and molecular changes. Recently, a comprehensive understanding of aging biology has emerged, encapsulated by the identification of nine molecular hallmarks of aging, including cellular senescence and depletion of stem cells [11]. These cellular alterations disrupt tissue homeostasis and regeneration, ultimately affecting organ function. Notably, in the quest to understand and address the challenges of aging, p16INK4a, a cell cycle inhibitor, has emerged as a promising candidate biomarker. Its potential to serve as a definitive biomarker for the complex process of aging opens up new avenues for research and intervention in the field of gerontology [12].

The INK4/ARF locus is a genetic region on human chromosome 9p21.3 [13] that spans over 35 kb. Within this locus, there is a gene called p16INK4a, which encodes a small ankyrin-repeat protein. This protein acts as an inhibitor of cyclin-dependent kinases. Together with INK4b (CDKN2B) and ARF (encoded by CDKN2A), p16INK4a is one of the three tumor-suppressor genes closely linked in this genetic region. By binding to CDK4 and CDK6 and inhibiting their activity, p16INK4a prevents the phosphorylation of retinoblastoma protein (RB) and causes cells to arrest in the G1 phase of the cell cycle. In addition to its role in regulating cell cycle progression and senescence, p16INK4a has also been associated with the repression of the hTERT gene. The exact mechanism by which p16INK4a regulates hTERT is still unclear, but it may

be worth investigating the possibility of p16INK4a directly binding to chromatin [14-17] and the molecular cloning of p16INK4a was achieved through its interaction with cyclin-dependent kinase 4 (CDK4). It was soon discovered that p16INK4a plays a crucial role in controlling the G1 phase of the cell cycle [18], acts as a significant factor in cell senescence [19], and is frequently deactivated in cancer [20]. Recent genome-wide association studies (GWAS) have further linked the INK4/ARF locus to various age-related conditions, including susceptibility to frailty and an increased risk of coronary artery disease (CAD), myocardial infarction, type 2 diabetes, and late-onset Alzheimer's disease. Interestingly, the INK4/ARF locus has been identified as the genetic locus most strongly associated with age-related pathologies [21]. Despite its involvement in cell cycle regulation, p16INK4a is not essential for early development and does not contribute to programmed developmental senescence [22,23]. Its expression is significantly suppressed during embryogenesis [24] and remains low in most somatic tissues of young mammals [25]. However, specific stresses, such as acute oncogene expression, effectively induce p16INK4a both in vivo and in tissue culture. As animals age, CDKN2A becomes increasingly expressed in most tissues, and the levels of p16INK4a serve as an indicator of age in both mice and humans [12]. Bioluminescence imaging in mouse models with a p16INK4a reporter also demonstrates an exponential increase in p16INK4a expression with aging [25,26].

What is the impact of p16INK4a on the aging process?

The protein p16INK4a plays a vital role in cellular senescence and the regulation of stem cells, which are both well-known indicators of aging [11]. Its active involvement in the intricate processes that govern aging emphasizes its importance beyond being a mere biomarker. As a crucial factor, p16INK4a contributes to the diverse array of elements that impact the aging process and the accompanying transformations that happen as time goes by.

p16ink4a: the maestro behind the aging symphony of cellular senescence

Cellular senescence was initially documented in 1961 by Leonard Hayflick and Paul Moorhead, who observed that human diploid fibroblasts lose their ability to divide after a certain number of passages in culture [27]. The maximum number of divisions that primary cells can undergo is known as the 'Hayflick limit', and it has been suggested that this limit is inversely correlated with the age of the donor in human fibroblasts. Replicative senescence is a common occurrence in most primary mammalian cells, except for embryonic stem cells (ESCs), which are known to be exempt from this phenomenon. Senescent cells exhibit a stable cell cycle arrest and undergo distinct changes in cell morphology, physiology, chromatin organization, gene expression, and the secretion of specific molecules [28]. This secretion, referred to as the senescence-associated secretory phenotype (SASP), allows senescent cells to influence their surrounding microenvironment and exert a significant local impact [29]. With age, senescent cells accumulate in various tissues including the liver, kidney, skin, lung, gastrointestinal tract, as well as components of the immune system such as hematopoietic stem cells (HSCs) and the spleen. This accumulation is associated with age-related conditions like atherosclerosis or osteoarthritis (as reviewed in [30]). Overall, the accumulation of senescent cells has detrimental effects on tissue homeostasis, contributing to the disruption observed during the aging process [11].

The implementation of senescence is heavily influenced by the two products of the CDKN2A gene, namely p16INK4a and ARF. These products control the retinoblastoma (Rb) and p53 tumor-suppressor pathways, with p16INK4a regulating Rb and ARF regulating p53. The extent to which these pathways contribute to senescence varies depending on the species and cell type [28].

During senescence in murine embryonic fibroblasts, both p16Ink4a and p19Arf (the murine equivalent of human p14ARF) accumulate. However, escape from senescence tends to occur through the loss of a functional Arf/p53 pathway [31]. This is evident in Arf-null MEFs, which are immortal, while Ink4a-null MEFs are not [32,33]. This suggests that Arf plays a more prominent role than Ink4a in MEF senescence. However, the role of these genes in senescence is cell type-dependent. In most human cells, p16Ink4a is considered the key regulator of senescence. This is supported by functional data and the frequency of inactivation during the establishment of immortal cell lines [34]. In primary human fibroblasts, replicative senescence is triggered by telomere shortening and derepression of the INK4/ARF locus. In other human cell types, replicative senescence is primarily driven by the progressive and powerful increase in p16INK4a expression during cell division [35]. Therefore, p16INK4a plays a crucial role in implementing senescence in response to excessive replication and other stresses.

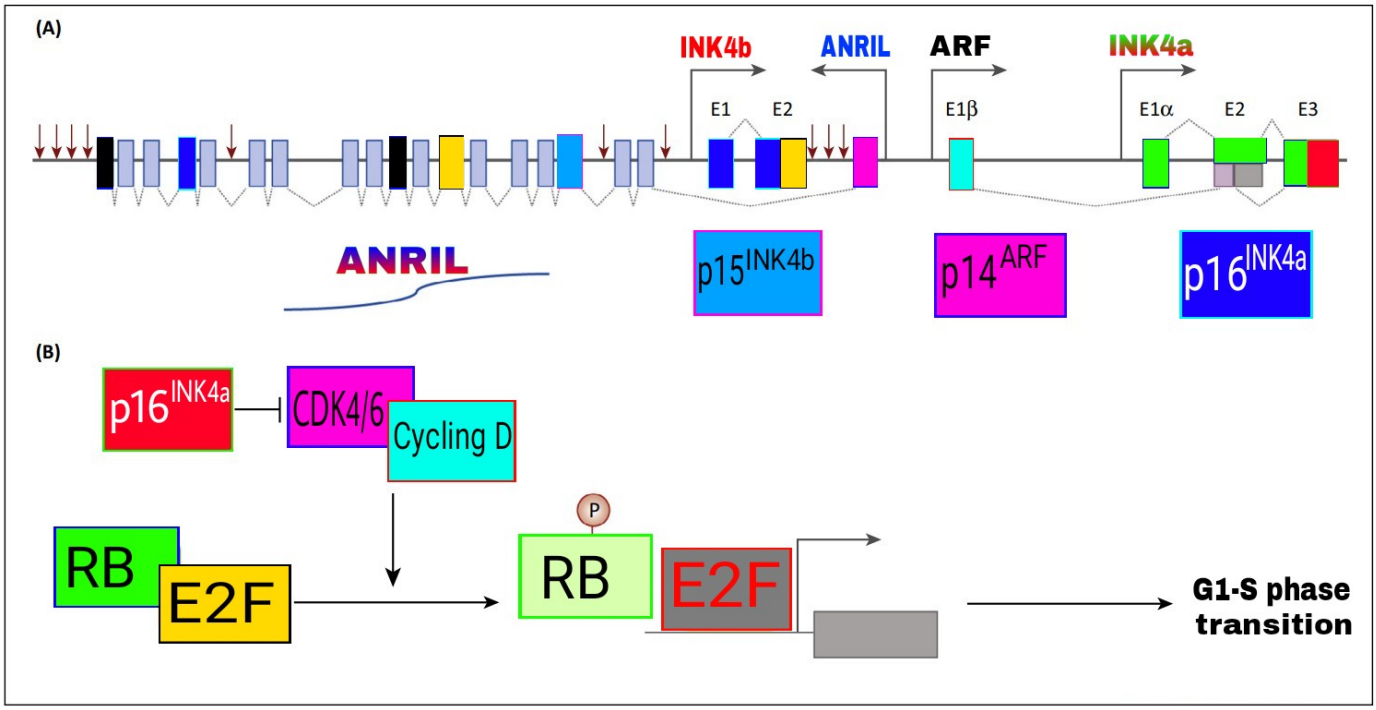

Fig-1 (A) The genetic structure of the INK4/ARF locus is illustrated, displaying coding exons for ARF, INK4a, and INK4b, as well as non-coding exons for ANRIL. Arrows denote single nucleotide polymorphisms (SNPs) linked to age-related disorders such as frailty, coronary artery disease, myocardial infarction, type 2 diabetes, and late-onset Alzheimer's disease. Please note that the map is not drawn to scale and the positions are approximate. **(B)** The inhibition of cyclin-dependent kinases CDK4 and CDK6 is facilitated by p16INK4a. This is crucial for preventing the phosphorylation of RB, which is necessary for the activation of E2F transcription factors that promote S-phase entry. By binding to CDK4 and CDK6, p16INK4a prevents their assembly with cyclin D, thereby blocking their activation and leading to cell cycle arrest in G1 [107].

Pathophysiology

Senescent cells (SnCs) display molecular characteristics such as the expression of senescence markers and morphological features such as an enlarged and flattened appearance that distinguish them from normal cells [36]. the phenotype of SnCs has generally enhanced the lysosomal biogenesis which further results in lysosomal hydrolase activity known as senescence-associated β-galactosidase (SA-β-gal) as well as the accumulation of cytoplasmic granules in the lysosomes and leading to an enlarged hypertrophic morphology [37, 38]. The initiation of the cellular senescence program is mediated by the p16INK4a through the Rb pathways and p53/p21CIP tumor suppressor pathways. p16INK4a, p21CIP1, and p53 are cyclin-dependent kinase inhibitors and tumor suppressors that work together to arrest the cell cycles in the G1 phase [39–41]. The expression of p16INK4a is known to increase in mammalian tissue with age which is a prominent marker of cellular senescence [42–44]. the expression of p16INK4a in CD3+ human peripheral blood T lymphocytes is a reliable indicator of chronological and biological age [45].

During senescence, there is an increase in the expression of p16INK4a and p21CIP1 in SnCs, which is accompanied by a decrease in lamin B1, indicating a disruption of the nuclear lamina [46]. This disruption is now considered a hallmark of SnCs [47]. Epigenetic changes in SnCs create an environment that allows for the uncontrolled activity of transposable elements like LINE-1, which is observed in late senescence [48,49]. Additionally, SnCs display DNA damage-associated characteristics such as DNA-SCARS and senescence-associated heterochromatin foci [50,51], which may play a role in determining the cell's fate decision for cellular senescence [52].

SnCs, despite being growth arrested in the cell cycle, exhibit metabolic activity [53]. A diverse range of bioactive substances, including inflammatory cytokines, chemokines, growth factors, matrix metalloproteinases, lipids, nucleotides, extracellular vesicles, and soluble factors, collectively known as the senescence-associated secretory phenotype (SASP), are secreted by numerous SnCs [54]. Furthermore, the SASP is influenced by various factors such as the release of DNA from the nucleus, referred to as cytoplasmic

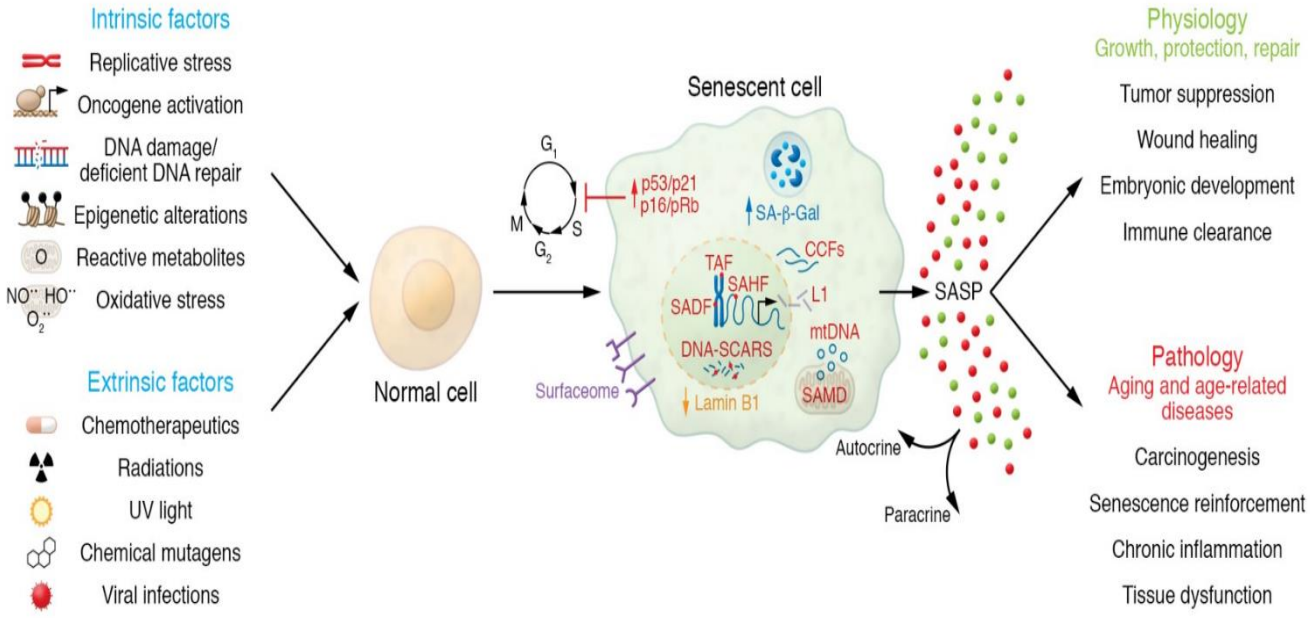

Fig-2 Diverse stress stimuli can trigger cellular senescence and result in the formation of senescent cells, which have multifaceted roles in both physiological processes and pathological conditions. CCF, cytoplasmic chromatin fragment; DNA-SCARS, DNA segments with chromatin alterations that reinforce senescence; mtDNA, mitochondrial DNA; SADF, senescence-associated DNA damage foci; SAHF, senescence-associated heterochromatin foci; SAMD, senescence-associated mitochondrial dysfunction; SASP, senescence-associated secretory phenotype; TAF, telomere-associated [108].

chromatin fragments, which occurs due to nuclear-cytoplasmic blebbing, mitochondrial DNA, NF-κB signalling, and the C/EBPβ transcription cofactor [55–57].

Cellular senescence is believed to have a mechanism to counteract tumor formation where the SASP induced by oncogene-induced senescence attracts immune cells to facilitate the removal of SnCs. SnCs are heterogeneous in nature and can arise throughout life due to various stimuli [58,59].

their heterogeneity of senescent cells (SnCs) also exhibits pleiotropic functions. SnCs play a crucial role in multiple physiological processes such as embryogenesis, cellular reprogramming, tissue regeneration, wound healing, immunosurveillance, and tumor suppression [60–66]. However, SnCs can also contribute to the pathology of numerous chronic diseases, including diabetes, cancer, osteoarthritis, and Alzheimer's disease [67, 68]. With age, SnCs tend to accumulate in most tissues, and the secretion of senescence-associated secretory phenotype (SASP) factors can induce secondary senescence both locally and distally, thereby creating a burden for SnCs [37, 54]. Moreover, the SASP also plays a role in sustaining and intensifying inflammation, a state characterized by chronic, low-grade systemic inflammation in the absence of pathogenic processes (69,70). Studies utilizing p16Ink4a-high senescent cell reporter mice (p16LUC and p16-CreERT2td Tomato mice) have demonstrated a progressive increase in p16Ink4a-expressing SnCs with age, which contributes to aging and cancer processes in mice [42, 71,72].

Additionally, in various mouse models of accelerated aging such as Ercc1–/Δ and Bub1bH/H hypomorphic mice, genomic instability leads to an accelerated accumulation of SnCs resulting in premature aging symptoms, including shortened lifespan and increased histopathological lesions in multiple organs [73–75].

The utilization of a transgenic p16Ink4a-expressing senescent cell removal system in mice known as INK-ATTAC, has provided additional evidence regarding the crucial role of SnCs in the process of aging and the development of diseases. This model allows for the selective elimination of SnCs through the use of the p16Ink4a promoter in combination with an FKBP-caspase-8 suicide transgene, which induces apoptosis of p16Ink4a-high SnCs via targeted activation of caspase-8 [76] with an FKBP dimerizer. The accumulation of SnCs not only contributes to the aging process but also plays a causal role in numerous age-related diseases, such as Alzheimer's, cardiovascular diseases, osteoporosis, diabetes, renal disease, and liver cirrhosis [77-81].

It is noted that the transplantation of a small number of SnCs into young and healthy animals reproduces age-related physical impairments [82, 83] supporting the threshold hypothesis which suggests that once the senescent cell burden exceeds the tissue's sustainability it triggers age-related pathological changes that eventually lead to disease. The genetic clearance of p16Ink4a-high SnCs in INK-ATTAC mouse models has demonstrated the benefits of SnC clearance in the prevention or alleviation of diseases, including osteoporosis, frailty, atherosclerosis, hepatic steatosis, osteoarthritis, idiopathic pulmonary fibrosis, obesity-induced anxiety, tau-mediated neurodegenerative disease, and type 2 diabetes mellitus/metabolic dysfunction [76, 84-87]. Furthermore, studies using the transgenic p16-3MR mouse model, which expresses luciferase and red fluorescent protein (RFP) reporters and herpes simplex virus-1 thymidine kinase, have shown that ganciclovir-induced genetic depletion of p16Ink4a-expressing SnCs alleviates multiple age-related dysfunctions [60, 88, 89]. Despite the use of a transgenic p16Ink4a-expressing SnC removal system in mice to study SnCs in aging and age-related diseases, the mouse model relies solely on the expression of p16Ink4.

This situation presents potential issues as not all cells expressing p16Ink4a are harmful, and some actually have physiological benefits [90]. The negative effects of senescent cells (SnCs) in aging and age-related diseases are likely caused by increased expression of senescence-associated secretory phenotype (SASP) factors [54, 91]. SASP factors, such as members of the TGF-β family, VEGF, and chemokines, are known to accelerate the accumulation of senescence by spreading it to neighboring cells [92,93]. The interaction between SASP and immune cells, including NK cells, macrophages, and T cells, exacerbates both local and systemic inflammation [94]. The proteases and growth factors present in the SASP are known to disrupt tissue microenvironments and promote the spread of cancer [54]. Fibrogenic factors and tissue remodelling factors in the SASP contribute to fibrosis in various tissues, including the skin, liver, kidney, lung, cardiac tissue, pancreas, and skeletal muscle [95]. Since the SASP disrupts tissue homeostasis and contributes to diseases, suppressing the SASP is an alternative approach to mitigating the harmful effects of SnCs in multiple studies.

For instance, inhibiting the SASP reduces inflammation, restores insulin sensitivity, mitigates osteoporosis, and improves physical functions in aged mice [96–99]. Eliminating the NF-κB-dependent SASP delayed the onset of progeroid symptoms and extended health span in Ercc1–/Δ mice [100]. Additionally, mTOR signaling also influences the SASP, and rapamycin, a selective inhibitor of mTOR complex 1 (mTORC1), significantly impairs the SASP, reduces inflammation, and extends health span and lifespan [101–104]. Overall, the accumulation of SnCs in aging tissues, coupled with the detrimental effects of the SASP, plays a significant role in driving the aging process and age-related diseases, thereby shortening both health span and lifespan. Importantly, removing SnCs or suppressing the SASP can alleviate or delay the onset of multiple chronic age-related conditions, highlighting the therapeutic potential of targeting SnCs [83, 105, 106].

Treatments

The deleterious effects of senescent cells (SnCs) in the aging process and various age-related diseases are likely caused by an increase in senescence-associated secretory phenotype (SASP) expression [54,91]. SASP factors, such as members of the TGF-β family, VEGF, and chemokines, have been shown to accelerate the accumulation of senescent cells by spreading senescence to neighboring cells [92,93].

The SASP also interacts with immune cells, including NK cells, macrophages, and T cells, exacerbating both local and systemic inflammation [94]. The presence of proteases and growth factors in the SASP has been found to disrupt tissue microenvironments and promote the metastasis of cancer [54]. Additionally, fibrogenic factors and tissue remodeling factors in the SASP contribute to fibrosis in multiple tissues, such as the skin, liver, kidney, lung, cardiac tissue, pancreas, and skeletal muscle [95].

Given that the SASP disrupts tissue homeostasis and contributes to various diseases, suppressing the SASP has emerged as an alternative strategy to alleviate the detrimental effects of SnCs in numerous studies. For instance, inhibiting the SASP has been shown to reduce inflammation, restore insulin sensitivity, mitigate osteoporosis, and improve physical functions in aged mice [96–99]. In Ercc1–/Δ mice, the abolition of NF-κB–dependent SASP delayed the onset of progeroid symptoms and extended health span [100]. Furthermore, mTOR signalling plays a role in modulating the SASP, and the selective inhibitor of mTOR complex 1 (mTORC1), rapamycin, significantly impairs the SASP, reduces inflammation and extends health span and lifespan [101–104].

Overall, the accumulation of SnCs in tissues with age, coupled with the detrimental effects of the SASP, plays a significant role in driving the aging process and age-related pathologies, ultimately shortening both health span and lifespan. Importantly, the removal of SnCs or suppression of the SASP has been shown to

alleviate or delay the onset of multiple chronic age-related conditions, highlighting the therapeutic potential of targeting SnCs [83,105,106].

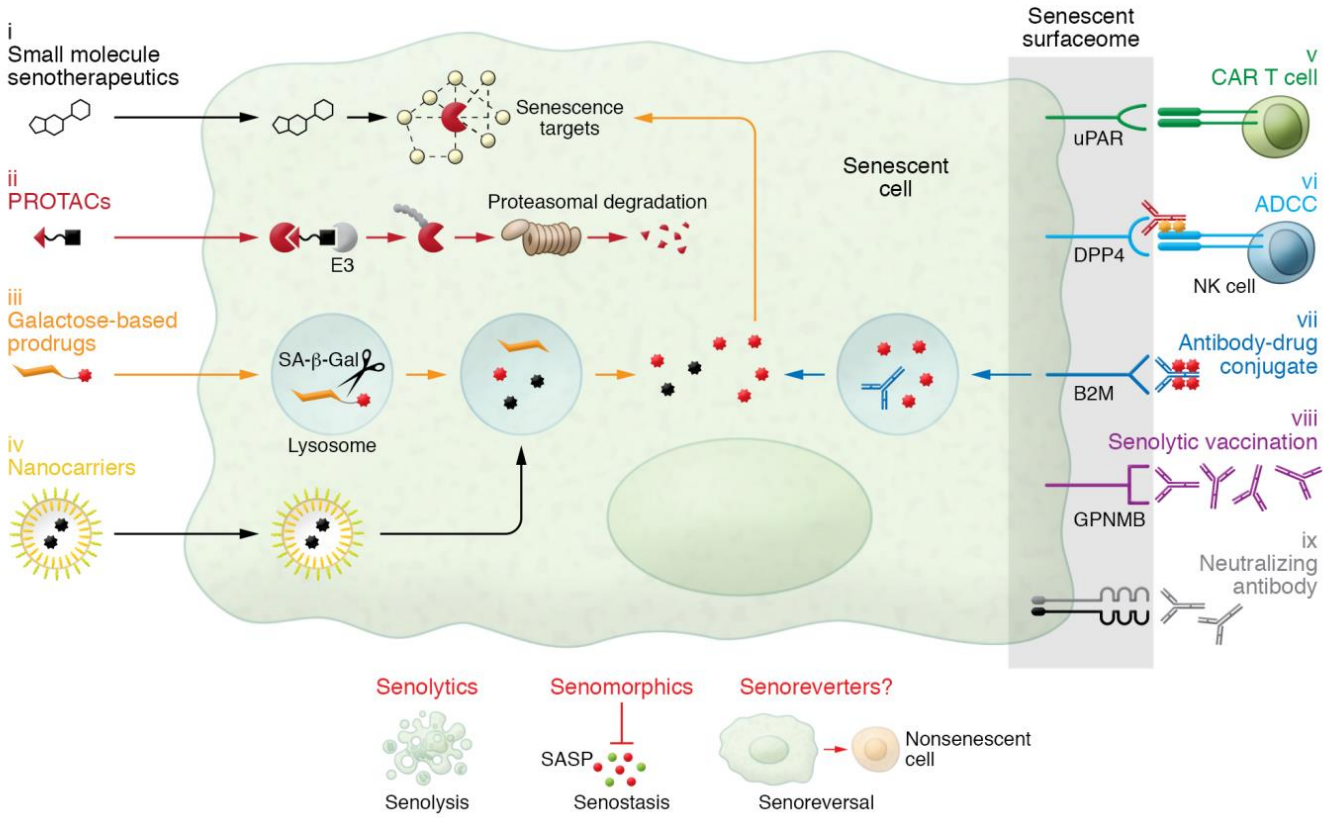

Fig-3. Current strategies to target senescent cells [108].

Revitalizing Aging Bodies: Innovative Approaches to Combatting Senescent Cells

As a result of the potential therapeutic benefits associated with reducing the burden of senescent cells (SnCs) to extend healthspan and delay the onset of age-related diseases, there has been a growing interest in the development of senotherapeutics that incorporate multidisciplinary technologies from various fields, including biology, chemistry, nanotechnology, and immunology [109-112]. Both intracellular senescence-associated pathways and extracellular membrane proteins upregulated on the surface of SnCs, known as the senescent surfaceome [113,114], can be utilized for therapeutic and diagnostic purposes (Figure 3). Current senotherapeutic strategies targeting SnCs include conventional senotherapeutics, prodrugs, protein degraders, nanocarriers, and immunotherapies.

Senolytics.

Senescent cells (SnCs) upregulate distinct antiapoptotic pathways known as SCAPs, which can be targeted for pharmacological interventions to promote senolysis. Various SCAPs and their key proteins have been identified as potential targets for drug development. The effectiveness of targeting SnCs was initially demonstrated using the senolytic combination of dasatinib plus quercetin (D+Q) [115]. Subsequently, several other senolytic agents have been reported, including inhibitors of antiapoptotic BCL-2 family proteins (such as navitoclax/ABT-263 and ABT-737), HSP90 inhibitors, USP7 inhibitors, p53 modulators (such as inhibitors of FOXO4-p53 or MDM2-p53 interactions), Na+/K+-ATPase inhibitors (such as cardiac glycosides), and

others [111,112]. Additionally, certain natural products like fisetin, piperlongumine, and curcumin have also been identified as senolytics, although their exact mechanisms of action remain unclear [111,112]. Currently, the two most extensively studied senolytics are D+Q and fisetin, both of which have entered clinical trials for the treatment of age-related diseases.

Senomorphics.

Compounds that mitigate the adverse consequences of the senescence-associated secretory phenotype (SASP) or inhibit senescence without causing cell death are referred to as senomorphics, also known as senostatics. Many episenomorphics function by interfering with transcriptional regulators of the SASP, such as inhibitors of ATM, p38 MAPK, JAK/STAT, and the NF-κB and mTOR pathways [111,112]. One potential drawback of senomorphics is that they likely necessitate continuous administration, unlike senolytics, which only require intermittent administration due to their hit-and-run mechanism [116]. Importantly, certain compounds have been found to exhibit both senolytic and senomorphic effects depending on the cell types and treatment concentrations. For example, procyanidin C1, a polyphenolic flavonoid derived from grape seed extract, acts as a senomorph at low concentrations but as a senolytic at higher concentrations [117].

Senoreverters.

Although cellular senescence is generally thought to be an irreversible cell fate, recent studies suggest that senescence in certain cell types is a dynamic process that can be reverted to allow SnCs to reenter the cell cycle [118,119]. For example, the suppression of NF-κB and mTOR signaling and inhibition of 3-phosphoinositide–dependent protein kinase 1 (PDK1) in senescent human dermal fibroblasts removed senescence hallmarks, and converted the cells from a senescent to a quiescent state, resulting in restored skin regeneration capacity [120]. Also, a specific six-factor gene cocktail reversed cellular senescence of senescent and centenarian fibroblasts and reprogrammed them into pluripotent stem cells [121]. Thus, senoreverters may provide a third therapeutic approach to target SnCs (Figure 3) [122]. However, there is also evidence that therapy-induced SnCs can escape the senescence state and acquire stemness features as well as more aggressive tumor growth potential through activated Wnt signaling [123]. Given that cellular senescence is a protective mechanism that suppresses tumorigenesis and metastasis [124], testing the safety of senoreverters will be extremely important.

WNT/b-catenin pathway

WNT signaling plays a crucial role in the regulation of various developmental processes, including primary embryonic axis formation, segmentation, organogenesis, and stem cell proliferation. Specifically, in the WNT/b-catenin pathway, extracellular WNT proteins bind to transmembrane receptors of the frizzled family, leading to the disruption of a cytoplasmic complex consisting of the GSK3b kinase and its substrate b-catenin. As a result, b-catenin accumulates and translocates to the nucleus, where it collaborates with TCF and LEF transcription factors to regulate gene expression [125]. Notably, the INK4apromoter contains a binding site for the b-catenin/LEF/TCF complex [126]. While b-catenin induces Arf in mouse cells [127], it has been observed to decrease proliferation in human colorectal cancer by inducing p16INK4a [126]. However, there are conflicting reports suggesting that activated b-catenin may repress, rather than activate, INK4a, thereby

immortalizing melanocytes [128]. This suggests that the role of b-catenin may be context-dependent. Supporting this notion, a genome-wide siRNA screen for p16INK4a modulators identified WNT3A as a repressor of p16INK4a [129].

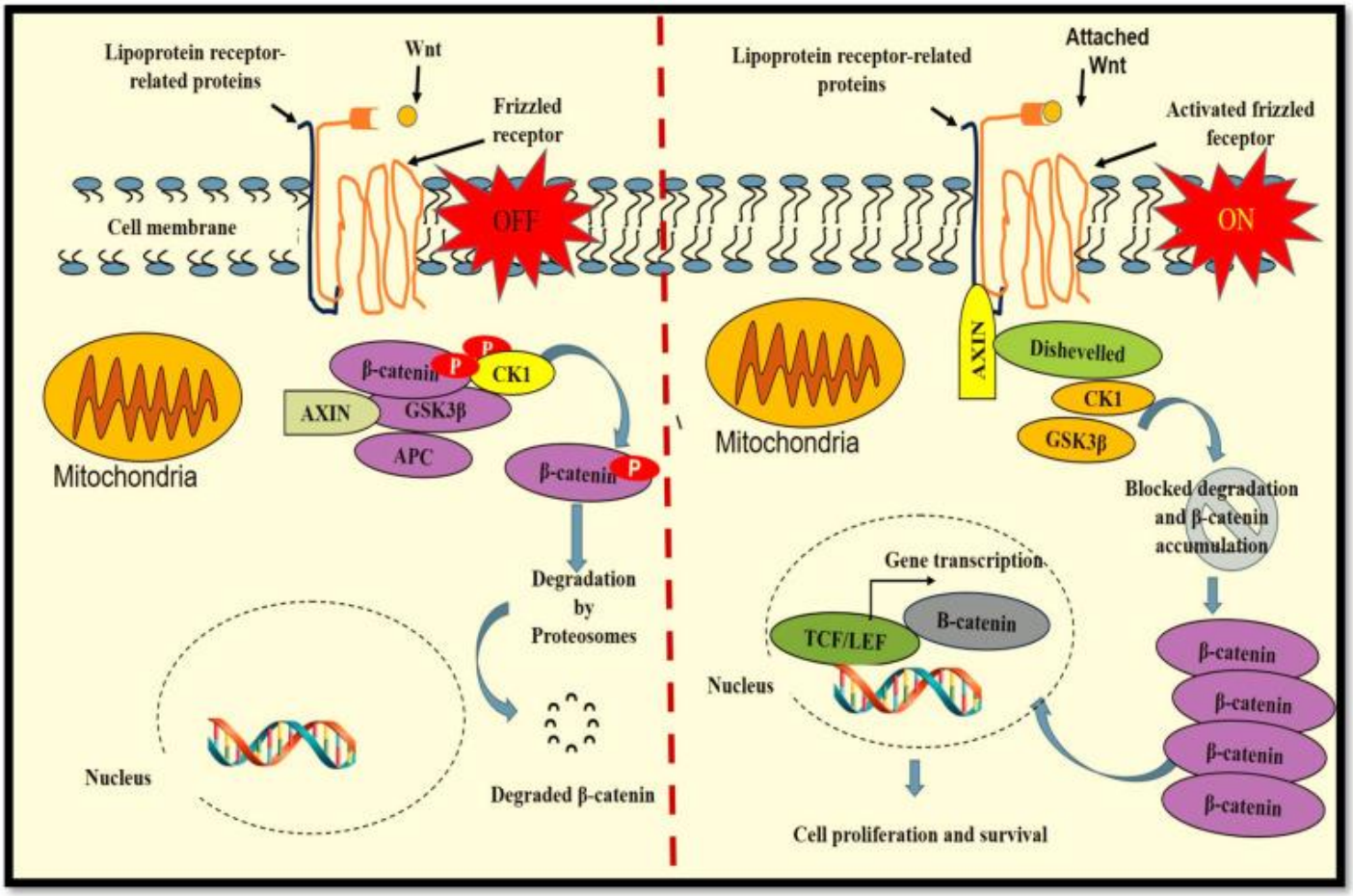

Fig-4: The Wnt/β-catenin pathway [130].

Conclusions and Future Perspectives

Senescent cells accumulate with age, and progeroid models have provided experimental data on accelerated aging-related degenerative pathologies. Furthermore, experimental senolysis has shown improvement in aging-related pathologies [131]. Therefore, cellular senescence meets the criteria for a potentially causal role in age-related diseases. There is evidence that cellular senescence affects astrocytes, microglia, oligodendrocyte progenitors, and neural stem cells. Post-mitotic cells have also demonstrated a senescence-like phenotype, suggesting that neurons and oligodendrocytes may also undergo senescence. Resident brain cells are either post-mitotic or slowly cycling, making them more susceptible to stress-induced premature senescence caused by various stressors or insults rather than replicative senescence. However, the evidence connecting cellular senescence with the mechanisms of neurodegeneration remains indirect, and further investigation into the potential role of senescence in neurodegeneration is necessary. The unequivocal identification of senescent cells in vitro and in vivo is an important prerequisite for enhancing our understanding of cellular senescence and its role in different cell types. The detection of lipofuscin as a marker of cellular senescence using the GL13 compound, which can detect lipofuscin both in situ and in biological

fluids [132], is likely to enhance our understanding of the senescence process. Shedding light on CNS cell senescence and its role in neurodegeneration is crucial for informing any practices that may induce senescence, such as the use of corticosteroids [133], beta-interferons [134], or DNA-damaging chemotherapeutics in MS, which may have detrimental effects in the long term. Additionally, there is an urgent need for disease-modifying treatments for neurodegenerative diseases. Cellular senescence may serve as a credible therapeutic target, opening up new avenues for neurodegenerative disease treatment, and senotherapeutics may prove to be effective neuroprotectants.

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