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Exploring the Role of Senescent Cells in Neurodegenerative Pathology: A Window Into Promising Therapeutic Avenues

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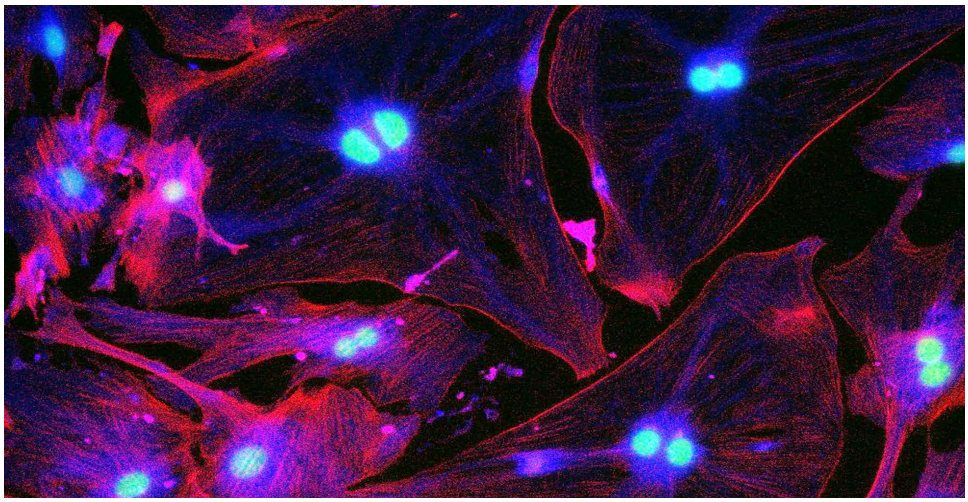

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Abstract

There is strong evidence that cellular senescence is involved in the pathogenesis of neurodegenerative diseases such as Alzheimer's and Parkinson's disease. Presence of abnormal tau protein in mouse models is implicated in senescence phenotypes leading to cognitive decline, while the significance of pro-inflammatory molecules called senescence-associated secretory phenotypes (SASPs) in inflammation and degeneration highlights its potential importance in disease treatment. Understanding the involvement of senescence in disease progression is vital to develop effective treatment strategies. Clearance of such cells can be performed through senolytics drugs that disturb non-apoptosis pathways. Alternatively, senomorphics involve the suppression of senescence burden, rather than elimination through apoptosis, through the intervention of SASP factors that elicit neuroinflammation and tau toxicity. Other therapeutic approaches involve targeting mitochondrial dysfunction to attenuate neuroinflammation and senescence stressors, thereby preventing the formation of a feedback loop. This review offers insights into the mechanisms of neuro-degeneration driven by cellular senescence in the brain and outlines novel therapeutic strategies to reduce the senescence burden and slow disease progression.

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1. Introduction

Cellular senescence is a state of irreversible growth arrest in cells that have aged and ceased their ability to multiply, yet they avoid undergoing apoptosis. This process is triggered in response to various stressors such as DNA damage, telomere shortening, mitochondrial dysfunction, tumor suppression, oxidative stress, and ionizing radiation (**Fig. 1A**). While senescence plays a protective role in development, aiding in wound healing and limiting tumor progression, its accumulation also leads to harmful effects associated with aging and age-related diseases. The harmful impact of senescence is mainly attributed to the release of SASPs. Research comparing biomarkers of senescence in young and aged mouse brains has shown that senescent cells increase with age, along with a rise in SASP factor genes.[1] Consequently, this contributes to decreased tissue regeneration and increased neuroinflammation, paving the way for neurodegenerative diseases like Alzheimer's disease (AD), Parkinson's, and multiple sclerosis. Furthermore, gene ontology analysis has revealed a strong connection between senescence-related genes, mild cognitive impairment, and neurodegenerative diseases.[2] Treating senescence has shown promise in improving cognitive function among patients with neurodegenerative diseases.

Glial cells are the primary cell type in the brain undergoing senescence, contributing to neurodegenerative diseases.[3] Glial cells play a supportive role in maintaining neuronal functions, and when compromised through senescence, they trigger SASP production, neurotoxicity, and neuroinflammation—common characteristics of neurodegenerative diseases.[4] Astrocytes, which are abundant in the brain and regulate synaptic signaling, neurotrophic support, and glutamate metabolism, have been found to be affected by senescence. Likewise, microglia, essential for CNS homeostasis, experience senescence during aging and are associated with neuroinflammatory phenotypes and neural impairment when increased in number.[1] Moreover, oligodendrocytes (OLG), derived from oligodendrocyte progenitor cells (OPCs), are involved in responding to neuronal injury and demyelination. In areas of the brain of AD patients, where there is elevated Aβ plaque and neural degeneration and

inflammation, there is a notable correlation with the presence of senescent OPC populations.[5] Considering the role of senescent glial cells in aging-related neurodegenerative diseases, targeting these cells has emerged as a potential therapeutic approach to tackle such conditions effectively. We may develop strategies to promote healthier aging and reduce the burden of neurodegenerative diseases.

2. Mechanisms of Senescence-Associated Neurodegeneration

In the realm of neurodegeneration, the intricate interplay between cellular senescence and the emergence of pathological hallmarks such as SASPs and tau pathology has garnered significant attention. These mechanisms not only shed light on the complex processes underlying aging-related neurological disorders but also offer insights into potential avenues for therapeutic interventions.

2.1 SASP

It was previously believed that senescent cells lack function; however, in reality they simply have altered important morphological functions.6 Many altered functions fall under SASP, which is a core feature of most senescent cells.[7] SASP is mainly characterized by the production and secretion of factors like cytokines, chemokines, and other molecules that create an inflammatory microenvironment.[6] The main function of SASP is to recruit immune cells for tissue damage repair; however, an abundance of senescent cells has a negative effect on tissue restoration and leads to a chronic, low-grade inflammation called "inflammaging".[8] Activation of inflammaging results in decreased clearance of senescent cells, establishing a positive feedback mechanism that ultimately further fuels inflammaging. In an autocrine or paracrine manner, senescent cells can secrete SASP factors that induce neighboring cells to senescence, furthering the accumulation of senescent cells (**Fig. 1B**). When this process occurs in the brain, it leads to the loss of neurons, resulting in the initiation, severity, and progression of neurodegeneration.[9]

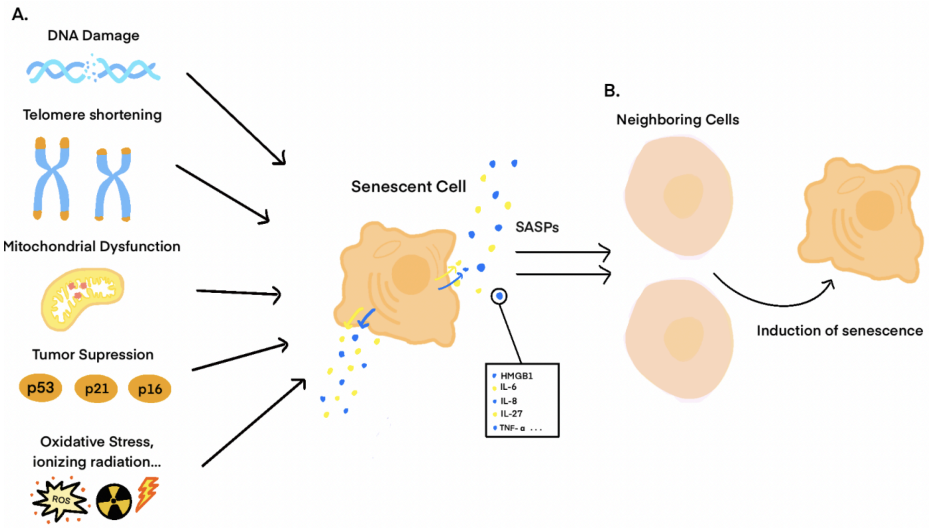

Figure 1: Mechanisms Triggering Cellular Senescence. (A) Cellular senescence is triggered by stressors such as DNA damage, telomere shortening, mitochondrial dysfunction, tumor suppression, oxidative stress, ionizing radiation, and many other abnormal factors. (B) Through the release of SASP factors, senescent cells can use autocrine or paracrine signaling to induce senescence in neighboring cells.

Early SASP is often induced by Transforming Growth Factor-β (TGF-β), secreted by senescent cells. TGF-β can also help maintain the secretory phenotype if its target cell is already senescent. If early SASP is upregulated for an extended period of time, it transforms into late SASP, which is regulated mainly through transcription factors CCAAT/enhancer-binding protein β (C/EBP β) and nuclear factor (NF)-κB. C/EBP β regulates the expression of various SASP factors, such as interleukin (IL)-1β, IL-6, and IL-8. Late SASP expression is induced when C/EBP β levels increase, suggesting C/EBP β is a key regulator of the early to late SASP transition.[7]

However, NF-κB signaling is the main signaling pathway in late SASP induction. NF-κB activation induces expression of inflammatory mediators IL-6, IL-8, and IL-1α. In a positive feedback loop, IL-1α contributes to SASP maintenance by regulating NF-κB and C/EBP β DNA binding activities to induce further IL-6 and IL-8 transcription. This feedback loop is regulated by rapamycin-mTOR signaling.[8] NF-κB signaling is also provoked by DNA damage through signaling pathways that include p38 mitogen-activated protein kinase (p38 MAPK) and retinoic acid inducible gene-1 (RIG-1).[10] p38 MAPK acts to stimulate NF-κB through the activation of MAPK-activated protein kinase 2 (MK2),[8] and p38 MAPK

signaling itself can be induced by environmental stress or chronic inflammation, such as inflammaging (**Fig. 2**).[10]

Persistent NF-κB activation is partially maintained by tumor necrosis factor (TNF)-α. Upon TNF-α activation, AK kinase is phosphorylated, which subsequently phosphorylates signal transducers and activators of transcription 3 (STAT3). These phosphorylated STAT3 molecules then migrate to the nucleus, contributing to prolonged activation of NF-κB and increased expression of SASP factors.[8] Interestingly, TNF-α itself is a common cytokine secreted by SASP.[4] Thus, its ability to induce SASP through NF-κB activation presents another positive feedback loop that serves to maintain SASP. Persistent SASP expression through NF-κB, as well as other contributing factors, induces proinflammation, which promotes tissue dysfunction, cellular aging, and other aging-associated issues (**Fig. 2**).[8]

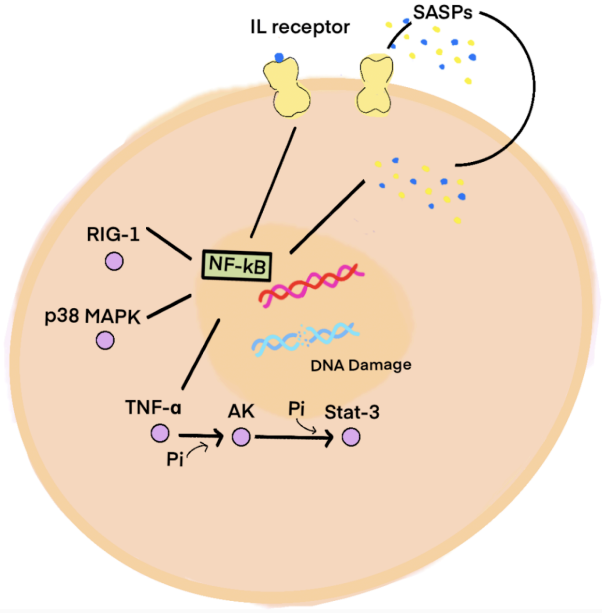

Figure 2: **SASP Regulation and Neuroinflammation Pathways**. Several different molecules act through the NF-κB pathway to activate and regulate SASP.

Upon acquisition of SASP, the secreted SASP factors promote autonomous neurotoxicity and disrupt normal function in the brain. In fact, it was found that treatment of senescent astrocytes with an antibody that neutralizes IL-6, a SASP-associated cytokine, alleviates neuronal cell death.[4] In vitro studies showed that IL-6 production was found to have the most dramatic increase in senescent astrocytes, matching in vivo findings of IL-6

at elevated levels in the CNS in AD patients.[6] Moreover, in a study with primary cortical neurons from senescence accelerated mouse prone 8 (SAMP8) mice, SASP factor plasminogen activator inhibitor 1 (PAI-1) was found to promote neuron apoptosis; however, the exact mechanisms by which PAI-1 carries out this activity is still unknown.[11]

Several SASP factors have been linked to axonal and myelin injury. Activation of SASP in neurons through the lymphotoxin-NF-κB pathway was found to trigger expression of chemokine CXCL13, which recruited CD8+ T cells. CD8+ T cells then activated caspase 3 in injured dorsal root ganglia (DRG), which was found to hinder signaling and lead to the repression of the neuron's axonal regeneration capabilities. However, the same study showed that regenerative failure in DRG could be restored by the neutralization of chemokine CXCL13 with monoclonal antibodies.[12] Similarly, myelin injury and regenerative failure can occur if SASP is activated in OLGs.[13] A study with mice showed that persistent NF-κB signaling in OLGs led to chronic neuroinflammation and post-mitotic senescence. NF-κB signaling also promoted white matter degeneration (WMD) in the brain, a well-known process of aging. WMD often manifests in myelin loss, which was confirmed by ultrastructural analysis of the mice that revealed impaired myelin protein expression. Additionally, the mice exhibited neurological deficits within three weeks following treatment.[14] OLGs have also been found to be affected by secretion factor high-mobility group box-1 (HMGB1) in vivo. When extracellular HMGB1 was released by senescent progenitor cells, differentiation of OPCs to myelinating OLGs was prevented, ultimately inhibiting CNS remyelination.[15] Moreover, HMGB1 prevailed at higher levels in the brains of patients with primary progressive multiple sclerosis (PPMS) compared to healthy controls. More specifically, HMGB1 was found at high concentrations in white matter lesions of the brain, and progenitor cells were identified to be the source.[16] This highlights the contributing role of SASP factor HMGB1 in the limited remyelination found in PPMS, as well as potentially other neurodegenerative diseases.

Examples of other neurotoxic SASP factors include IL-8, IL-1, matrix metalloproteinases, insulin-like growth factors (IGFs), endothelial growth

factor (EGF), epidermal growth factor receptor (EGFR), specific miRNAs, and many other molecules.[7]

2.2 Biomarkers

Understanding the relationship between cellular senescence and neurodegenerative conditions is crucial for developing effective therapeutic strategies. To this end, researchers have focused on identifying biomarkers that link cellular senescence to neurodegeneration. Several enzymes, cytokines, chemokines, genes, and other biomarkers have been implicated in senescence-related processes in the brain. These biomarkers provide valuable insights into the mechanisms underlying neurodegenerative diseases and offer potential targets for therapeutic interventions.

Enzymes

- Senescence-Associated Beta-Galactosidase (SA-β-gal): SA-β-gal is one of the earliest markers identified for the detection of senescent cells in situ within tissues.[7] This hydrolytic enzyme becomes active in senescent cells and is commonly used as a hallmark to distinguish senescent cells from non-senescent cells.
- Caspase 3: Caspase 3 is an enzyme involved in apoptosis. It suggests a potential link between senescence and cell death pathways.[18]

Cytokines

Cytokines are signaling molecules that play critical roles in inflammation and immune responses. Certain cytokines have been found to be associated with the development of neurodegeneration.

- IL27: This cytokine is involved in the activation of Natural killer cells in the dentate gyrus, which leads to the elimination of neuroblasts and subsequently contributes to neurodegeneration and cognitive decline.[19]
- IL-6, IL-8, IL-1β: IL-6, IL-8, and IL-1β are among the cytokines that mediate tumor suppressor functions. Studies conducted by Bussian et al. and Musi et al. have identified these cytokines in the brains of mouse models with tauopathy. They were found to be part of the SASPs observed in these models.[20,21]

Chemokines

Chemokines are small signaling proteins involved in cellular migration and immune responses. One chemokine that has been linked to senescence-associated neurodegeneration is CCL2.[22]

- CCL2: Studies have implicated CCL2 in the development of neurodegenerative processes. It may contribute to the recruitment of immune cells, such as microglia, to the site of neurodegeneration, further promoting inflammation and tissue damage.23 Apart from its role in immune cell recruitment, CCL2 can also directly influence neuronal function. It has been suggested that CCL2 might affect synaptic plasticity and neurotransmission, potentially contributing to cognitive and functional impairments seen in neurodegenerative diseases.[22]

Genes

Several genes have been identified as crucial regulators of cellular senescence, and their dysregulation may contribute to neurodegeneration.

- p16, p21, p53: These genes encode cell cycle inhibitors and repressors and are essential components of the senescence program. Exposure to tau has been shown to increase the expression of senescence-associated markers, including p16INK4a and p21W AF1, in microglia, potentially contributing to neurodegeneration.[24]
- CDKN2A and CDKN2D/p19: Neurons expressing CDKN2D/p19, a gene related to CDKN2A, were found to be more prone to containing tau aggregates and displaying signs of neurodegeneration, suggesting a potential role for senescent neurons in the development of tauopathy.[25] Additionally, NFTs (neurofibrillary tangles) have been directly linked to CDKN2A upregulation.[21]

Other Biomarkers

Other biomarkers associated with senescent cells and neurodegeneration have been identified.

- B2M: B2M is an extracellular epitope of senescent cells and serves as a membrane marker for MHC class I molecules. Its expression may contribute to immune responses and inflammation in the context of senescence.[26]
- PAI-1: Plasminogen activator inhibitor-1 is a serine protease inhibitor that increases with aging and has been found to contribute to brain cell senescence. However, the specific mechanisms linking PAI-1 to neurodegeneration are still being investigated.[11]
- HMGB1: High mobility group box 1 serves as a crucial biomarker. This multifunctional protein plays significant roles within the cell, particularly in DNA repair and autophagy regulation. Studies conducted by Gaikwad et al. and Rouilliard et al. have brought to light its potential implications in the advancement of neurodegenerative diseases. HMGB1's involvement as part of the SASP is particularly noteworthy, as it contributes to neuroinflammation and impedes the growth of myelin, which is essential for nerve fiber insulation. [15,27]
- Lamin B1: Lamin B1 is a protein that plays a crucial role in maintaining the structural integrity of the cell nucleus. It is a component of the nuclear lamina, a network of proteins that provides support to the nuclear envelope. Lamin B1 has been implicated in the aging process as its expression levels decline with age in various tissues.28 Reduced levels of Lamin B1 have been observed in certain senescent cells, and its loss has been linked to nuclear envelope disorganization and altered gene expression, contributing to cellular dysfunction and aging-related changes.[28]
- Igfbp5: Insulin-like Growth Factor Binding Protein 5 is a member of the insulin-like growth factor-binding protein family and plays a role in regulating the bioavailability and activity of insulin-like growth factors (IGFs). IGFs are essential for cell growth, proliferation, and survival. Igfbp5 modulates the actions of IGFs by binding to them and regulating their interactions with cell surface receptors.[29] Additionally, Igfbp5 has been found to be involved in processes related to brain development and function, and its dysregulation may influence neuronal survival and synaptic plasticity, potentially contributing to neurodegeneration.[29]

2.3. Tau Pathology

The tau protein, predominantly located within neurons, plays a vital role in maintaining the structural integrity of nerve cells by stabilizing microtubules and facilitating efficient axonal transport.[4] In addition to its structural functions, tau protein is involved in essential cellular processes such as cell signaling, synaptic plasticity, and the preservation of genomic stability, and in OLGs cells, it also plays the role of myelination.[30,31] When tau becomes abnormal, it can contribute to the development of neurodegenerative diseases. Excessive phosphorylation, genetic mutations, and mis-splicing contribute to the abnormal aggregation of tau protein, resulting in the formation of either tau oligomers or neurofibrillary tangles (NFTs).[32] Tau oligomers are small aggregates of abnormal tau protein that form prior to the development of NFTs, which are larger insoluble clumps of hyperphosphorylated tau protein. Both tau oligomers and NFTs contribute to pathogenesis of neurodegenerative diseases. Abnormal tau can be secreted by neurons into extracellular space, which are taken up by healthy neighboring neuronal and glial cells through endocytosis, pinocytosis, or phagocytosis means, thereby contributing to the propagation of tau pathology across interconnected brain regions.[27] In addition, extracellular tau was found to induce human astrocyte senescence, inflammation, and SASPs. The inflammatory state can cause induction of senescence in nearby cells in a paracrine-like manner, further propagating neurodegeneration, which is why overall extracellular tau may be more neurotoxic than intracellular tau.[4] One pathological change caused by abnormal tau accumulation is the disruption of cell function and the blood brain barrier (BBB), which is responsible for protecting the CNS from pathogens and maintaining homeostasis in the microenvironment of the brain.[31] Other tau pathologies include chronic neuroinflammation, DNA damage, and oxidative stress, which are known stressors of senescence. These tau-related pathologies contribute to the establishment of a vicious cycle, where tau pathology triggers senescence, leading to the release of toxic SASP factors that, in turn, exacerbate the formation of abnormal tau (**Fig. 3**).

Neurons

Senescent cells are present in both AD and non-AD brains. However, it has been determined that the number of senescent cells was significantly higher in AD brains. Additionally, a subset of senescent cells in AD brains were neurons that expressed the gene CDKN2D/p19. These neurons also showed signs of tau neuropathology.[25] Moreover, an examination of the transcriptomic profile of cortical neurons containing neurofibrillary tangles (NFTs) in AD brains revealed that those neurons displayed a senescence-like phenotype. This means that the gene expression patterns observed in these neurons resemble those typically seen in senescent cells, suggesting that AD neurons undergo a cellular aging process akin to senescence.[21]

Astrocytes

Tau can be transmitted from neurons to astrocytes, causing the astrocytes to senesce and produce SASPs. Studies have shown that astrocyte senescence is detrimental to dendritic and synaptic structure and density. This suggests that pathogenic soluble tau-induced astrocyte senescence may contribute to synaptic dysfunction and loss in AD.[33] Recent research has highlighted that tau oligomers play a role in promoting the release of HMGB1 from astrocytes during inflammation. Notably, the release of HMGB1 not only leads to inflammation but also drives paracrine senescence in neighboring cells.[27] Moreover, tau oligomers have been specifically implicated in promoting the aggregation of p53 in AD patients. In the study, this interaction between tau oligomers and p53 was observed exclusively in AD brains, compared to control, resulting in the sequestration or aggregation of p53 outside the nucleus. Consequently, this phenomenon hinders the ability of p53 to carry out DNA repair processes, leading to DNA damage and ultimately triggering senescence. The accumulation of senescent cells further exacerbates the progression of AD.[34] One study found that astrocytes and microglia are the two major types of senescent cells that accumulate in the dentate gyrus of the hippocampus in AD.[20] This was supported by the laboratory of Human Carcinogenesis, where they found that astrocytes are the primary type of senescent cells present in patients with AD, amyotrophic lateral sclerosis (ALS), aged individuals and those receiving cranial radiation.[4] Flow cytometry analysis of PS19 mouse models, which overexpress human mutant tau, also validated these findings by

revealing increased expression of senescence-associated genes, including p16Ink4a, in isolated astrocytes and microglia, while OLGs and neurons did not exhibit the same upregulation. Furthermore, the study reported that senescent astrocytes and microglia exhibit inflammatory reactions to extracellular tau, leading to damaged neurons.[20] In the context of neuronal damage, it has been identified that astrocyte senescence is associated with the downregulation of glutamate and potassium transporter genes. Consequently, this impairment of glutamate clearance increased susceptibility of neurons to glutamate-induced toxicity and ultimately neuronal cell death (**Fig. 3**).[35]

Microglial

In the aged human brain, many microglial cells show morphological features indicative of senescence or degeneration rather than activation. This suggests that microglial senescence and associated loss of neuroprotection could be involved in aging-related neurodegenerative diseases. In fact, Streit et al. reports that dystrophic microglial cells are implicated in the development of AD, associated with severe tau pathology. Investigation of microglial dysfunction by examining their morphology in the vicinity of tau-positive structures revealed that microglial degeneration may precede the onset of NFT pathology, meaning that the loss of microglial structural integrity contributes to neurodegeneration.[36] Karabag et al. conducted a study demonstrating that exposure to tau can induce microglial senescence. Specifically, they found that an exposure to 15 nM concentration of tau resulted in increased levels of cell cycle arrest and a DNA damage marker. It also led to the loss of nuclear envelope protein lamin B1 and the histone marker H3K9me3, thereby affecting the clearance of tau and impairing cell migration (**Fig.3**). Additionally, this exposure caused alterations in cell morphology and triggered the formation of a SASP.[24]

Oligodendrocytes

Tauopathies involving OLGs have been identified in specific dementias, such as progressive supranuclear palsy (PSP), corticobasal degeneration, and frontotemporal dementia, which includes Pick's disease and certain cases of AD.[37] Because pathological tau has the ability to propagate from neurons to other cell types both trans-cellularly and trans-synaptically, it can contribute

to the spread of neurodegenerative disease.[38] However, studies now propose that OLGs can contribute to the spread of tau without passing through intra-neuronal transport in a glial-to-glial fashion, playing a further role in the spreading of tauopathies.[39] The spread of tau pathology to OLGs is closely linked to the loss of high-firing pyramidal neurons, a crucial neuron type essential for cognitive function. The depletion of these neurons can lead to cognitive decline, as assessed through various behavioral tests in mice. In one study, mice injected with human tau aggregates displayed prolonged search times in locating the platform in the Morris water maze, reduced visits to the correct arm in the Y -maze, and decreased exploration time of the novel object compared to control mice.[37] The aggregation of tau in OLGs may be a contributing factor to the development of gait abnormalities and myelin loss in neurodegenerative diseases (**Fig. 3**).[30] In fact, Luo et al., found that OLG lineage cells from old donors had lower myelination potential than OLG lineage cells from younger donors.[40]

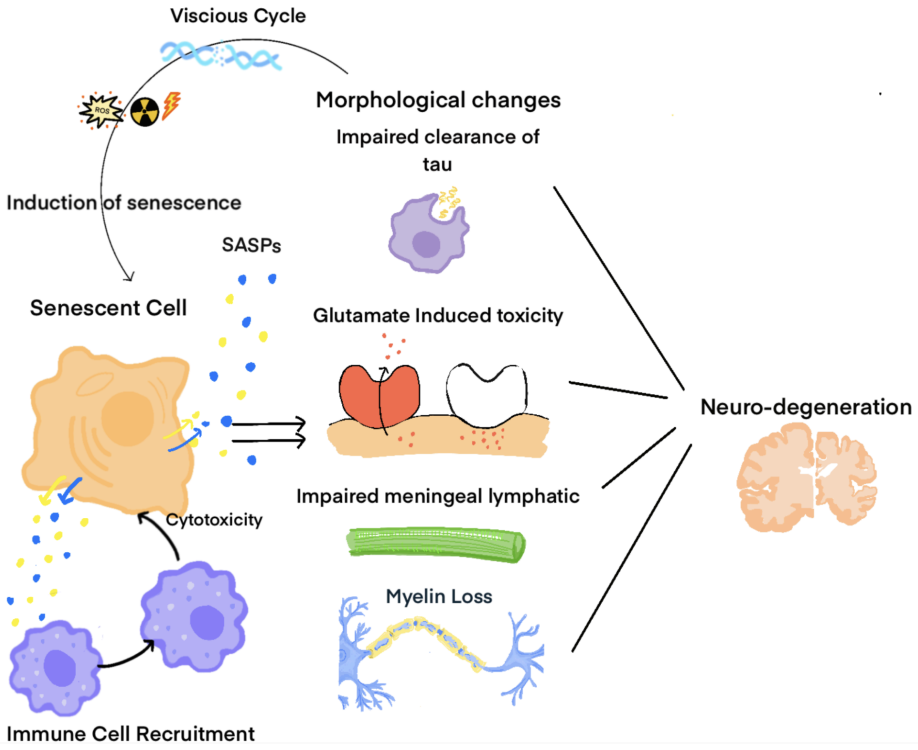

Figure 3: Cellular Morphological Changes in Senescence. Senescent cells undergo morphological changes, such as altered cell size and structure, that lead to neurodegeneration. These cellular changes make up a vicious cycle as they contribute to the propagation of other senescent cells.

3. Therapeutic Approaches

3.1. Senolytics

Senolytics eliminate senescent cells by inducing apoptosis. This is done by targeting senescent cell anti-apoptotic pathways (SCAPs) such as p53 and BCL-2. Through removing senescence, senolytics have the potential to reduce neuroinflammation, enhance tissue regeneration, and improve cognitive function. The effects of senolytics are currently mainly tested through mouse models and cell populations characterized with an age-related disease.

Dasatinib and Quercetin

The combination of dasatinib and quercetin (D+Q) is one of the most extensively studied senolytics, shown to be effective in numerous age-related diseases by selectively targeting senescent cells while maintaining the viability of non-senescent cells. Dasatinib is a tyrosine kinase inhibitor that was initially discovered for use in cancer treatments to prevent cancer cell growth. By targeting pro-survival pathways, dasatinib induces apoptosis to senescent cells. Quercetin is a natural flavonoid and enhances the effectiveness of dasatinib with its anti-inflammatory and pro-apoptotic properties. When used together, their effectiveness as a senolytic increases, as they are able to target a larger variety of senescent cell types. Beyond targeting SCAPs, quercetin has also been found to improve cognitive function in AD patients through activating AMP-activated protein kinase leading to a reduction in Aβ-induced mitochondrial dysfunction, a main characteristic in early AD pathogenesis.[41] Through clearance of senescent cells by targeting BCL-2 and other anti-apoptotic proteins, D+Q has shown to be effective in numerous age-related conditions. By decreasing senescence, D+Q relieves tissue degeneration, inflammation, and other SASP factors.[42] D+Q acts in a dosage-dependent manner positively correlating with increased senescent cell death in mice with AD, specifically in OLGs. These mice were also found with reduced levels of Aβ plaque associated proinflammatory cytokines such as Il-1β5. NFTs are also reduced by D+Q in tau transgenic mice with effects of improved cerebral blood flow and

decreased neurodegeneration.[21] Intermittent treatment with D+Q on mice showed improved cognitive function by reducing senescent microglia. Y oung and aged mice were put through phenotypic, memory, and cognitive assessments. Assessment results of young mice were unchanged while aged mice improved significantly in a Stone T-maze following treatment of D+Q suggesting high effectiveness of D+Q during aging.[43] A current clinical trial of D+Q involving early-stage older AD patients suggests promising results. The patients were treated intermittently with D+Q for 12 weeks while their cognitive function and progression of AD were measured. The only statistically significant changes in safety parameters of D+Q observed was an increase in total cholesterol, yet this higher total cholesterol remained in a normal range, and one possibly related adverse event, yet this event was resolved between 1 to 16 days. Several tests were conducted to test changes in cognitive function including the Montreal Cognitive Assessment (MoCA) and the Clinical Dementia Rating Sum of Boxes (CDR SOB). There was no significant change in the results from these cognitive assessments from the baseline to post-treatment. MRIs also showed no significant change from the baseline to post-treatment, suggesting a stable brain morphology over the treatment period. Plasma and cerebrospinal fluid (CSF) levels revealed that D was able to penetrate through the BBB while Q was not able to. Overall, the study's results are promising in the safety and potential of D+Q as a senolytic, yet further studies are needed to examine long-term efficacy.[44]

Fisetin

With studies of quercetin, a natural flavonoid, being an effective senolytic, other flavonoids were explored for their senolytic abilities such as fisetin. Fisetin, present in various fruits and vegetables, was found to possess great seno-therapeutic qualities.[45] It is assumed that with similar chemical structures, fisetin and quercetin hold similar therapeutic and anti-inflammatory effects.[46] With a fisetin diet, abundance of p16 was reduced in aged mice and levels of p16 and SASP factors continued to remain in significantly low levels following treatment. Senescence was reduced in multiple organs of the mice along with markers of inflammation and oxidative stress.[45] Through blocking mitogen activated protein kinase (MAPK) and NF-κB signaling pathways, fisetin is able to reduce

inflammation.[46] Specifically, fisetin targets senescence through the SCAP networks of BCL-xL and HIF-α. Fisetin has also shown to be able to extend median and maximal lifespan, suggesting promising results for age-related treatments.[47] Fisetin also attacks the P13K/AKT pathway, which promotes cell survival, to induce apoptosis.[48] Human adipose tissue explants treated with fisetin had a decrease in SA-β-gal positive cells and SASP expression of IL-6, IL-8, and MCP-1. Similar to D+Q, fisetin was effective in reducing senescence without affecting proliferating cells.[45] Currently, fisetin has proven to be successful as a senotherapeutic agent, yet further testing is needed to study its safety for age-related and neurodegenerative treatments.[47]

Navitoclax

Navitoclax, also known as ABT263, is another discovered senolytic. Initially used as an anti-cancer drug, Navitoclax works by inducing apoptosis in cancer cells. This mechanism is likewise in its role as a senolytic drug, disrupting BCL-2 and pro-death proteins to induce apoptosis in senescent cells.[49] This disruption is caused by Navitoclax binding to BH3 of BCL-2, resulting in the displacement of a pro-apoptotic protein, BIM, that causes cell apoptosis (**Fig. 4**).[50] Aged mice treated with Navitoclax enhanced neurovascular coupling, regulating cerebral blood flow that is essential for cognitive function. Examination through a radial arms water maze, Navitoclax improved on the learning capabilities and memory retention in aged mice.[51] Hippocampal neurogenesis reduced by aging can be restored by Navitoclax. By targeting senescent cells that impair neural progenitor cells (NPCs), hippocampal neurons and hippocampus-dependent spatial memory function was increased following Navitoclax treatment.[52] Trails of Navitoclax were tested on p16 transgenic mice in biweekly cycles. Apoptosis of senescent cells increased in these mice, yet the possibility of thrombocytopenia and neutropenia as side effects rose.[48]

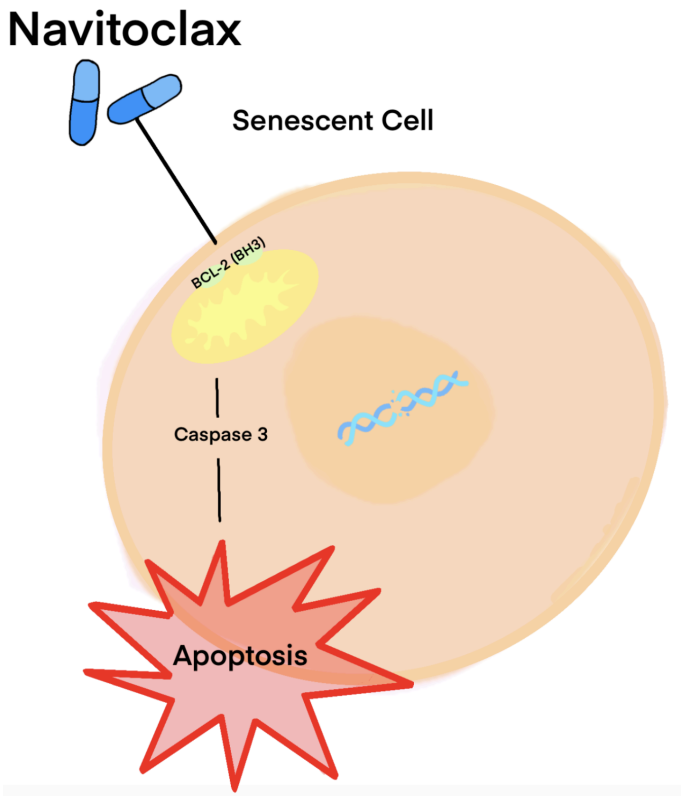

Figure 4: Navitoclax Mechanism of Action in Senescence. The figure demonstrates Navitoclax targeting the BCL-2 pathway to release Caspase 3 and cause apoptosis in senescence.

AP-20187

AP-20187 (AP) is a drug that targets senescence by disrupting SCAPs and activating a pro-apoptosis protein.[53] A study on young and old INK-A TTAC mice revealed significant effectiveness in clearing senescence during aging following intermittent treatment of AP. Cognitive impairment was reduced without affecting the physicality of the mice. Specifically, aging-induced senescent microglia was reduced in aged mice with AP treatment.[43] In another study, AP increased neuron density and decreased tau aggregation in PS19 mice, which express models of tauopathy, with twice-weekly administrations. With treatment, expression of senescent genes were measured at levels compared to that of control mice. Treatment of AP was able to mitigate short-term memory loss in novel-scent discrimination assessments.[20]

Limitations

Senolytics is an emerging field and therefore there remains a lack of information on the exact mechanisms of senolytics in neurodegenerative diseases and long-term effects. Future studies are necessary to test the safety and efficacy of senolytics long-term before being able to be used beyond clinical trials.[54] While senolytics have shown to improve lifespan, cognitive functions, and anti-inflammation, senolytics have the risk of eliminating benefits of senescence such as wound healing and tissue development. Since senescent cells are not replaced when removed by senolytics, fibrosis can occur, resulting in scarring and thickening of tissues.[55] As there are other cell types that are non-senescent yet can have a high p16 expression, some senolytics such as Navitoclax and AP can have off-target effects and eliminate non-senescent cells.[43,56]

3.2. Senomorphics

Senomorphic medications intervene with SASP factors without inducing apoptosis. Unlike senolytics, the benefit of senomorphic drugs is that they do not kill functioning senescent cells, which are still important for anti-tumor cell signaling (**Fig. 5**). Senomorphic medication mainly targets signaling pathways: NF-κB, mTOR, IL-1α, p38 MAPK, etc. There are currently multiple senomorphic drugs being tested to varying levels of success that will be covered in the following sections.

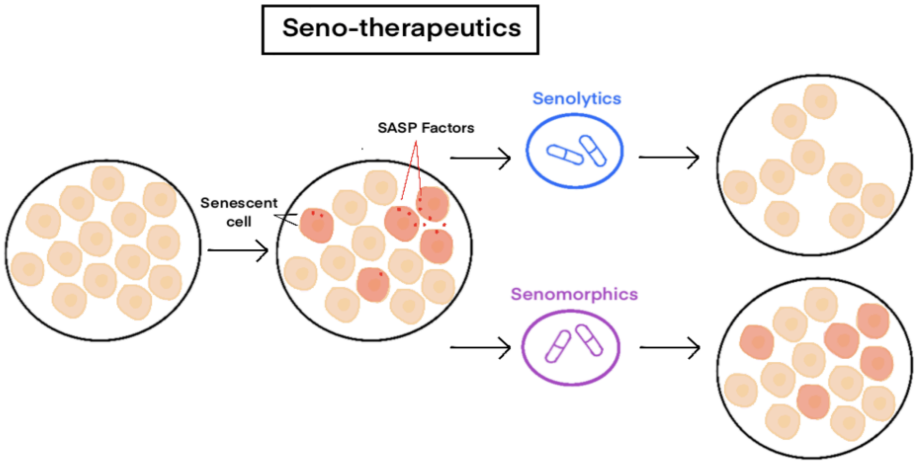

Figure 5: Comparison of Senolytic and Senomorphic Mechanism of Action and Effect. Senolytics eliminate senescent cells, while senomorphics modulate SASP factors to mitigate inflammation without inducing apoptosis.

Metformin

Metformin has a neuroprotective effect in animal models, and is shown to reduce inflammation and oxidative stress.[57] Metformin is a drug that was originally approved for the treatment of Type 2 diabetes, but its therapeutic effects have expanded into age-related disorders and neurodegenerative diseases. It is effective in suppressing cellular senescence and SASPS; for example, Metformin reduced cellular SA-β-gal activity and down-regulated the expression of senescence and SASP factors in human diploid fibroblasts—cells commonly found in the perivascular spaces, meninges, and choroid plexus of the brain—among other cells.[58,59] Metformin also increases the lifespan of different model organisms, including mice.[60] Despite the fact that Metformin has been tested in detail, its exact mechanism of action remains unknown, and is an area for further experimentation and testing. Metformin has been proven to reduce senescence via transcriptional up-regulation of Nrf2-medicated GPx7 in human diploid fibroblasts, but it also decreases cellular senescence and SASPS via the microRNA processing protein DICER1.[61,62] Metformin has been found to influence all hallmarks of aging, which is why understanding its mechanism of action is so complicated. Its impact has been found in nutrient signaling pathways, insulin signaling, repairing oxidative damage, and inhibiting protein synthesis, among others.[63] Currently, there is an ongoing clinical trial (NCT04098666) for Alzheimer's prevention using Metformin. At baseline and after study visit at 18 months a brain MRI, physical exams, plasma tau and amyloid beta levels are being measured to assess prevention of AD, along with clinical interviews, physical exams, and brain MRIs. Results are inconclusive as of yet.[57]

Resveratrol

Resveratrol is an SIRT1 activator that plays an important role in promoting anti-inflammatory and antioxidant properties in the body, and has an effect on cellular senescence.[8] It also mitigates neuroinflammation by promoting microglia polarization towards the M2 phenotype it activates, PGC-1α: a transcription factor involved in the suppression of SASP.[64] M2 microglia are

involved in anti-inflammatory responses; they promote tissue repair and protect neurons from damage; thus, promoting the M2 phenotype will help reduce neuroinflammation and protect neurons from further damage. In trials, long term Resveratrol treatment prevented age-dependent decline in cellular viability and various cell parameters—metabolic, oxidative, inflammatory, and senescent—in hypothalamic astrocytes from aged rats. It also increased the expression of genes involved in cellular homeostasis, such as: Nrf2, HO-1, SIRT1, and PGC-1α.[65,66] However, Resveratrol has a biphasic effect. At low concentrations (below 10 μM), Resveratrol acts as a senomorphic and prevents cellular senescence and suppresses SASPs. For example, Resveratrol prevented cellular senescence by activating the telomerase of endothelial progenitor cells via the P13K-Akt pathway.[67] The drug has also been shown to suppress SASP factors by inhibiting NF-κB and upregulating Nrf2 pathways in the vascular smooth muscle cells of rhesus monkeys. However, at higher concentrations (over 25 μM), Resveratrol triggers growth arrest and induces senescence of apoptotic death in cell lines. As such, controlling the dosage of Resveratrol is very important for using it as a senomorphic therapy.

Rapamycin

Rapamycin is also known as sirolimus. While it was originally used as an immunosuppressive drug for the prevention of organ rejection in kidney transplantation, studies have shown that rapamycin could reduce cellular senescence and suppress SASP markers in a variety of mouse and human cell lines. It is an incredibly well-established senomorphic drug. In multiple invertebrate models, including yeast, flies, and worms, rapamycin has increased lifespan, and late-life administration has extended lifespan in male and female mice. In vivo studies have demonstrated that rapamycin alleviates age-related dysfunctions, decreases aging rate, and increases life-span. Further, Rapamycin treatment has reversed the cellular senescence phenotype in PPMS NPCs, as evidenced by the reduced expression of senescence markers and increased cellular proliferation. It further enhanced the PPMS NPC support for OLG maturation, as seen in the increased expression of OLG differentiation markers and decreased HMGB1 secretion.[16] Decreased HMGB1 secretion is important; it is seen that patients with PMS exhibit higher levels of HMGB1, a senescence marker,

which inhibits the differentiation of progenitor cells into OLGs. This is incredibly important for neurodegenerative disease progression, as OLGs are what produce myelin to protect our neurons.16 Rapamycin has side-effects, however: metabolic dysregulation and impaired wound healing, among others. Rapamycin works by inhibiting TORC1 activity in association with the intracellular protein FKBP12. TORC1, or Target of Rapamycin Complex 1, is a eukaryotic protein complex related to cell proliferation.[68] The senomorphic and longevity effects of rapamycin relate to its inhibition of mTOR signaling by reducing the phosphorylation of S6K and 4E-BP. Other potential or secondary mechanisms by which rapamycin may regulate senescence and lifespan are being explored—for example, activating the Nrf2 pathway and decreasing NF-κB activity to reduce IL-1α production. It is also to be determined whether other mTOR pathway inhibitors and rapalogues such as everolimus, temsirolimus, deforolimus, ridaforolimus and zotarolimus may exhibit senomorphic activities.[63]

Aspirin

Aspirin has been found to partially prevent Aβ-induced neuronal senescence and DNA damage by upregulating sirtuin-1 (SIRT1) in a dosage-dependent manner.[23] It also delayed the onset of senescence in endothelial cells by increasing nitric oxide synthesis and decreasing oxidative stress, subsequently upregulating telomerase activity.[69] However, other experimental studies indicate that the effects of aspirin on senescence vary with context, stress type, and dosage.[63]

Ethyl Pyruvate and Glycyrrhizic Acid

Ethyl Pyruvate and Glycyrrhizic Acid inhibit the release of HMGB1, and can also inhibit p53 aggregation and prevent tau phosphorylation.[27,34] This indicates that the two drugs could have potential benefits in treating Alzheimer's. Other areas that are only just being explored include NF-κB inhibition, p38 MAPK inhibition, The Janus kinase/signal transducer signaling pathway, mutated Ataxia telangiectasia inhibitors, and statins.[63] Overall, senomorphic medications are a very promising area of research for treatment of neurodegenerative diseases, but there is still a lot of work to be done and discoveries to be made.

2.3. Mitochondria-Based Therapies

Mitochondria are the dominant sources of reactive oxygen species (ROS), which is produced as a by-product of the electron transport chain in mitochondria. Under normal conditions, ROS is central for organismal homeostasis, able to promote necessary signal transduction by mediating redox modifications of specific molecules; however, damaged mitochondria often result in an overproduction of this superoxide, leading to oxidative stress, another leading factor of cellular senescence. Imbalances between ROS production and detoxification of these reactive species, or antioxidant activity, is toxic to cells. It activates p53, which induces the cyclin-dependent kinase (CDK) inhibitor p21, causing cell cycle arrest and senescence. In addition, elevated ROS levels have also been found to be strong modulators of inflammatory pathways that can accelerate cellular senescence and further aggravate inflammaging. As a result, dysfunctional mitochondria have become a hallmark of and contributor to aging and aging-related neurodegeneration. While the role of dysfunctional mitochondria in aging and cellular senescence is still being explored, many studies show that therapies targeting these defects have been effective in alleviating senescence-associated symptoms.[7]

NAD+ supplementation

NAD+ supplementation has rapidly become a popular research avenue as a potential therapy for neurodegenerative diseases with underlying mitochondrial dysfunction-induced senescence causes. Studies have shown that NADH improves motor symptoms in Parkinson's disease and NAD+ supplementation can prevent STING-induced senescence in A-T cells and mice, while promoting mitophagy to remove damaged mitochondria and prevent senescence and neuroinflammation.[70,71] Additionally, the cGAS-STING pathway has been implicated in attenuating neuroinflammation, suggesting that boosting NAD+ with nicotinamide riboside (NR) treatment could serve as a therapeutic approach to mitigate neurodegeneration.[72] These findings highlight the potential of NAD+ as a therapeutic strategy for addressing senescence-related issues in neurodegenerative diseases.

Limitations

Studies have shown that increased NAD+ levels could lead to the accumulation of potential toxic metabolites and may even contribute to tumorigenesis.[70] Additionally, excessive NAD+ supplementation in young healthy mice, which already have sufficient NAD+ levels, has been linked to elevated levels of pro-inflammatory cytokines such as IL1β and IL6, suggesting unintended consequences on inflammatory pathways.[71] These findings highlight the need for cautious consideration of NAD+ supplementation as a therapeutic approach, with attention to dosage and potential age-related variations to avoid adverse effects. Further research is necessary to fully understand the risks and benefits of NAD+ supplementation as a therapy for senescence and neurodegenerative diseases.

MAPKs

Mitogen-activated protein kinase 15 (MAPK15) is another potential target of mitochondria-based therapies. MAPK15, an atypical mitogen-activated protein (MAP) kinase, has been found to control the mitophagic process by stimulating phosphorylation of Unc-51 Like Autophagy Activating Kinase 1 (ULK1)-dependent parkin RBR E3 ubiquitin protein ligase (PRKN) Ser108, which induces recruitment of damaged mitochondria to lysosomal compartments for disposal. As such, by effectively getting rid of defective mitochondria, MAPK15 helps prevent oxidative stress and DNA damage accumulation, both of which are effective in inducing senescence. In a study conducted with human Airway Epithelial Cells, it was found that the downregulation of MAPK15 resulted in reduced cell proliferation, increased p21 levels, increased SA-β-Gal activity, and increased expression of SASP cytokines, all of which are associated with senescence.[73] The usefulness of other members of the MAP kinase family is still being investigated as they have a duality that both helps prevent and promote the negative effects of senescence. The activation of p38 MAPK has been observed in senescent cells indicating an involvement in the cellular senescence process. In an α-synuclein mouse model of Parkinson's disease it has been shown to promote mitochondrial fission. Chemical inhibition of p38 MAP with molecule SB203580 was shown to protect cells from mitochondrial dysfunction and cell death.[74] Additionally, p38 MAPK plays

a crucial role in the secretion of SASP factors. Interestingly, p38 MAPK can induce SASP independently of the DNA damage response (DDR), suggesting its significance as a separate pathway for SASP activation. Thus, when p38 MAPK is inhibited, it has been found to effectively suppress the SASP , potentially offering a promising target for therapeutic interventions aimed at mitigating the harmful effects associated with cellular senescence.[75] Furthermore, it is interesting to note that MAPKs and NF-κB have been found to regulate the expression of the other in a feedback mechanism, suggesting inhibition could lead to regulation of SASPs.[76,77] Further research on p38 MAPK's intricate role in senescence and its modulation may provide valuable insights into aging-related processes and age-associated diseases. While specific therapies that target MAPKs have not yet been thoroughly developed, these findings point to the great potential of MAPK-based treatments.

Limitations

Excessive and constitutive mitophagy may contribute notably to the progression of neurodegenerative disease. In addition, in vitro studies on chronic obstructive pulmonary disease (CPOD) epithelial cells found that cigarette smoke activated MAPK15 signaling and led to oxidative stress, rather than the opposite, as what has been more commonly discovered in other studies.[78] Hence, patients with neurodegenerative diseases that smoke may encounter additional barriers to treatment with MAPK15-targeted therapies.The p38 MAPK has also been linked to oxidative stress, playing a role in the induction of senescence. Thus caution of these therapies is essential in order to avoid producing the opposite effect.

4. Future Directions

It is crucial to explore the heterogeneity of senescent cells and their distinct contributions to neurodegeneration. Understanding the specific factors and signaling pathways that drive the detrimental effects of different senescent cells in neurodegenerative diseases will help guide the development of targeted therapies. One area that could be further explored is the use of targeted therapies. Some research has been done but needs to be expanded upon for applications in neurodegenerative diseases. One of those is

second-generation senolytics: they offer a specific approach to enhance the effectiveness of existing senolytic drugs while minimizing the side effects. An example is B2M ADC, an antibody conjugation that increases efficacy and selectivity when it comes to using senolytics to clear senescent cells.[79] Future research in combining strategies, like using senolytic drugs and immunotherapy simultaneously promises potential benefits as well. Similar to how CAR T cell therapy has been used to treat cancers, this therapy can be applied and engineered to target senescent biomarkers for neurodegenerative diseases. So far very limited research has been done in this area but Amor et al. successfully created a senolytic CAR T therapy that effectively eliminated senescent cells in mouse models and improved outcomes in liver fibrosis. However, This technique comes with its limitations. A condition called cytokine release syndrome may arise, in which an intense storm of T cells causes fever and impacts breathing and blood pressure.[80] In addition, antibodies made to neutralize SASPs are another promising avenue to take in the path of immunotherapy treatments. Particularly, intervention of cytokine IL27 as well as the upregulation of MHC class-I-related molecules, such as RAE1 can be applied to alleviate senescence burden and interaction between NK cells and receptor NKG2D, which influences age-related decline in neurogenesis and cognition.[19]

Another area of research is in leveraging the lymphatic system, which plays an imperative role in clearing senescent cells and therefore preventing neurodegenerative diseases. Such diseases are known to cause chronic inflammatory cascades that damage hippocampal micro vessels and therefore lymphatic drainage. This in turn creates a dangerous cycle where the disease causes lymphatic blockage of senescent cell clearance and senescent cells clearance accelerates cognitive decline that further damages hippocampus. A study determined that such drainage is dependent on the VEGF-C/CCL21 pathway. Leveraging these pathways to improve lymphatic drainage could prove extremely beneficial in combination with senolytics like D+Q. One such idea is dispersing Astrocyte VEGF-C into the CSF promoting lymphangiogenesis, and thus enhancing drainage of parenchymal waste to the dural lymphatics. Given that studies have demonstrated the aggravation of pathogenic tau accumulation in AD

mouse models when AQP4 water channels are pharmacologically inhibited, there is a need for further research strategies for mitigating the downregulation of AQP4 water channels in senescent astrocytes. These channels play a crucial role in facilitating cerebrospinal fluid and interstitial fluid exchange, thereby regulating glymphatic transport and helping clear senescent cells.[23,35] Overall, further research into second generation-senolytics, immunotherapy, and lymphatic drainage can pave the way for improved therapies to alleviate senescence burden and neurodegenerative disease onset and progression.

5. Conclusion

The field of neurodegenerative diseases is only just beginning to be explored; the brain is a complicated area of study, and cognitive decline even moreso. In recent years, many potential avenues have been explored for possible treatments, including senolytics, senomorphics, and mitochondrial-targeted therapies. Senescence is a vital part of neurodegenerative diseases, and understanding its role proposes a powerful avenue of treatment. Constant stimuli, such as NF-κB signaling, contributes to SASP , reinforcing inflammaging by its characteristic overproduction of proinflammatory cytokines and chemokines.[8] In addition, it is clear that many factors function to induce and maintain SASP in a positive feedback manner, increasing senescent cell accumulation and exacerbating chronic inflammation. These factors contribute to a neurotoxic environment in which neuronal cell death occurs, advancing neurodegeneration in affected patients. Extracellular tau can further cause oxidative stress and DNA damage, which are known to be associated with induction of senescence.[4] In addition, therapeutic strategies targeting senescence offer promising avenues for addressing neurodegenerative diseases. Senolytics have shown potential in reducing neuroinflammation and improving cognitive function, while senomorphics provide alternatives by modulating SASP factors without inducing cell death. Similarly, mitochondria-based therapies hold promise in mitigating oxidative stress and enhancing cellular health. However, challenges remain in understanding mechanisms, potential side effects, and disease-specific applications. Collaborative research across disciplines is vital

to fully harness the potential of these approaches and reshape the landscape of neurodegenerative disease treatment.

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