Book Chapter

The link Between Senescence and Alpha-Synucleinopathy in Microglia and Astrocytes Encourages the Therapeutic Use of Senolytics in Parkinson's Disease

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Abstract

Parkinson's disease (PD) is the most common movement disorder and the second most prevalent neurodegenerative disease after Alzheimer's disease. Despite decades of research, there is still no cure for PD and the complicated intricacies of the pathology are still being worked out. Much of the research on PD has focused on neurons, since the disease is characterized by neurodegeneration. However, neuroglia has become recognized as key players in the health and disease of the central nervous system. This review provides a current perspective on the interactive roles that α-synuclein and neuroglial senescence have in PD. The self-amplifying and cyclical nature of oxidative stress, neuroinflammation, α-synucleinopathy, neuroglial

senescence, neuroglial chronic activation and neurodegeneration will be discussed. Finally, the compelling role that senolytics could play as a therapeutic avenue for PD is explored and encouraged.

Keywords

Parkinson's disease; Senescence; α-Synuclein; Astrocyte; Microglia

Introduction

Parkinson's disease (PD) is the most common neurodegenerative disease that is primarily associated with the loss of motor function. It is also the second most prevalent neurodegenerative disease besides Alzheimer's disease. Although PD is considered primarily a movement disorder, it can present with severe non-motor symptoms such as impaired bladder control, sleep disturbances, emotional disturbances, and constipation. The risk for PD increases with age, male gender, pesticide exposure, and melanoma [1-3]. Conversely, PD risk has an inverse relationship with nicotine use, caffeine intake, and urate levels [2,4-6]. Several gene mutations have been associated with increased risk. Familial PD is linked to such genes as SNCA, PRKN, LRRK2, PINK1, FBX07, PLA2G6, and others [7]. Sporadic PD cases have been associated with genetic mutations in genes such as GBA, ACMSD, STK39, NMD3, STBD1, GPNMB, FGF20, MMP16, STX1B, ITGA8, and others [8]. The underlying pathophysiology of PD is linked to oxidative stress and inflammation [9,10]. Recent and compelling discussions have also highlighted the role of lipidopathy in PD pathology [11]. However, proteinopathy is the pathological hallmark of the disease, as it is in many neurodegenerative diseases.

The primary focus of neurodegenerative pathophysiology has been historically centered on the role of misfolded pathogenetic proteins. For example, amyloid-beta peptides are implicated in Alzheimer's disease, TAR DNA-binding protein 43 is implicated in amyotrophic lateral sclerosis and frontotemporal lobar degeneration, the huntingtin protein is implicated in

Huntington's disease, and α-synuclein is implicated in PD [12-16]. Furthermore, many neurodegenerative diseases involve aggregation of the tau protein, including Alzheimer's disease, amyotrophic lateral sclerosis, frontotemporal lobar degeneration, and PD [17,18].

Aging is the greatest risk factor for developing PD [19]. The nine classic hallmarks of cellular aging include genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and finally, altered intercellular communication, which is linked to chronic inflammation [20]. The Geroscience Hypothesis identifies the following seven "pillars of aging": macromolecular damage, epigenetics, inflammation, adaptation to stress, proteostasis, stem cells and regeneration, and metabolism [21].

Senescent cells are intrinsically linked to the aging process. The senescence-associated secretory phenotype (SASP) of senescent cells releases pro-inflammatory cytokines, chemokines, and promotes inflammation [22]. The recently developed Unitary Theory of Fundamental Aging Mechanisms describes the cellular facets of aging as being so closely interrelated that therapeutic targeting of one aspect, such as cellular senescence, might mitigate many, or all, of the others [23]. Furthermore, the Unitary Theory of Fundamental Aging Mechanisms identifies several other additional aging-related hallmarks such as increased fibrosis, increased CD38, decreased $NAD^{+,}$ and the accumulation of misfolded and aggregated proteins [23].

Operating under the Unitary Theory of Fundamental Aging Mechanisms, a direct relationship should exist between senescent cells and proteinopathy. Since most of the research conducted on senescent cells has focused on peripheral tissues, it is especially of interest to explore the relationship between senescent cells and proteinopathy in the central nervous system (CNS) [24]. Here, we explore the relationship between α-synucleinopathy, senescent astrocytes, and senescent microglia in PD. Additionally, the potential of senolytics for therapy in PD will be discussed.

Background

Introduction to α-Synucleinopathy in PD

The protein α-synuclein is small (14 kDa), soluble, intrinsically unstructured, and encoded by the *SNCA* gene [25]. The intrinsically disordered nature of monomeric α-synuclein is stable and conserved across mammalian cell types [26]. α-synuclein is located ubiquitously in CNS presynaptic terminals [27]. Although the normal function of α-synuclein is not well understood, it is known to be involved in some regulatory roles such as neurotransmitter release and synaptic plasticity, dopamine metabolism, membrane remodeling, and DNA repair [28-30]. The most common α-synuclein isoform found in humans is 140 amino acids long [27,31].

Under normal physiological conditions, the structure of α-synuclein resists aggregation. The N-terminal region is amphipathic, has a basic pH, binds to membranes, and changes from a disordered structure to an α-helical structure when bound to lipids [32,33]. The N-terminal region spans the first 60 residues of α-synuclein and is the location of three familial PD mutations: A30P, E46K, and A53T [34]. The N-terminal region also includes the beginning of a stretch of seven imperfect repeats of "KTEKEGV" [35]. N-terminal acetylation destabilizes α-synuclein, increases α-synuclein levels, and enhances α-synuclein toxicity [36]. The central core spans from residues 61 to 95 and consists of hydrophobic amino acids. The central region is also referred to as the non-amyloid-β component (NAC) and is the site essential for misfolding and aggregation [34]. In wild-type α-synuclein, the NAC is protected from cytoplasmic exposure due to long-range interactions between the N-terminal and the C-terminal, which acts to prevent aggregation [26,37]. Additionally, chaperones are known to bind to the N-terminus around tyr39, which further helps to prevent aggregation [38]. Mutations in the N-terminal have been shown to disrupt the interaction between the N-terminal and the C-terminal to promote the pathological gain-of-function α-synuclein aggregation [37]. The remainder of the imperfect "KTEKEGV" repeated motifs are found in the NAD region. The

C-terminal is intrinsically disordered and is highly acidic [39]. The structure of α-synuclein is depicted in Figure 1.

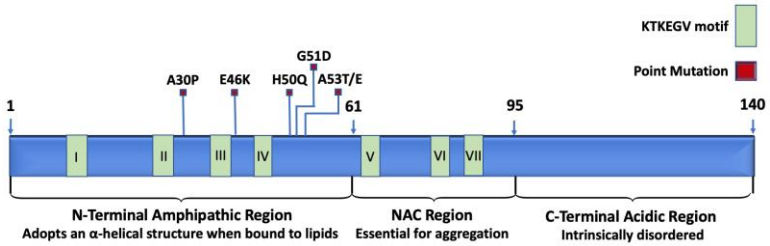


Figure 1: Schematic representation of relevant landmarks on the protein α-synuclein. The N-terminus is characterized by being amphipathic, containing mutations associated with familial PD, four of the seven KTKEGV repeats, and lipid-binding sites that induce the disordered structure to adopt an α-helical secondary structure when bound to lipid membranes. The central non-amyloid-β component (NAC) is hydrophobic and is the site where α-synuclein proteins interact with each other to form aggregates. The C-terminus is acidic and intrinsically disordered.

The *SNCA* gene in humans has a chromosomal location of 4q22.1, a length of 114,226 base pairs, and contains six exons [40]. *SNCA* transcription is regulated by beta-2-adrenoreceptor (B2AR), which can be antagonized to increase the risk of PD or activated to reduce the risk of PD [41]. The first genetic mutation identified to be associated with PD is the G-to-A transition at the 209th nucleotide, which results in the A53T mutation of the *SNCA* gene located between the 4th and 5th repeat of KTEKEGV [16,42,43]. The familial and highly penetrant A53T mutation is inherited in an autosomal dominant manner and is associated with early-onset PD [44]. In rat dopaminergic PC12 cells, the A53T mutation has been shown to induce α-synuclein related cell death due to reduced proteasome activity, increased reactive oxygen species (ROS), increased mitochondrial permeability and dysfunction, cytochrome C release, increased activity of caspase-3, caspase-9, and caspase-12, and finally, endoplasmic reticulum (ER) stress due to α-synuclein accumulation in the ER [45-47].

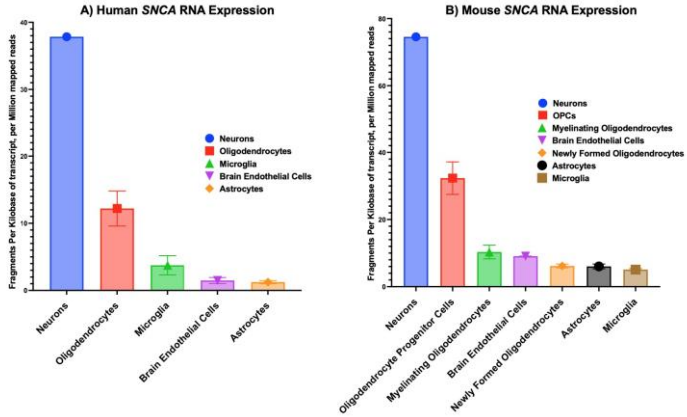


Figure 2: Cellular Distribution in the Central Nervous System of *SNCA* RNA Expression. (A) Human cellular distribution of SNCA transcripts. (B) Mus musculus cellular distribution of SNCA transcripts. RNA-Seq data used for these graphs were obtained and adapted from the Ben Barres lab's brain RNA-seq database, www.brainrnaseq.org. The original mouse data came from [289] and the original human data came from [290].

Sequence mutations in the *SNCA* gene, such as A53T, are known to increase the rate and magnitude of α-synuclein aggregation. Subsequent to intracell α-synuclein aggregation, dopamine accumulates in the cytoplasm and dopaminergic toxicity increases in severity [48]. Duplications or triplications of wild-type *SNCA* is also implicated in PD pathology, PD with dementia, dementia with Lewy bodies and multiple system atrophy [49]. *SNCA* triplication is linked to early-onset autosomal dominant familial PD and PD-related dementia, as demonstrated in the Spellman-Muenter kindred, a Swedish-American family, a family from Italy, and several others [50,53]. Human carriers of the triplicate mutation of *SNCA* had twice the control levels of α-synuclein mRNA in blood and brain tissue [54]. Soluble α-synuclein protein levels were also doubled in the blood of the triplicate *SNCA* carriers, whereas the genomic triplication of *SNCA* led to greater levels of insoluble α-synuclein aggregates in the brain [54]. DA neurons differentiated from human-induced pluripotent stem cells (hiPSCs) from a *PARK4* patient who also had *SNCA* triplication showed increased levels of α-synuclein compared to control hiPSC-derived DA neurons [55]. Genomic duplication of *SNCA* also increases α-synuclein levels and is causal for familial PD [56,57]. There is

well established direct relationship between *SNCA* copy number, α-synuclein abundance, and PD phenotype severity [58]. In contrast to triplication, cases of *SNCA* duplication resemble idiopathic PD with a late onset, slow progression, and are spared from dementia [56,57]. However, there has been a single case described recently of a male with *SNCA* duplication who developed early onset PD with aggressively progression and rapid cognitive decline [59].

The abnormal accumulation of phosphorylated α-synuclein into insoluble aggregate is characteristic of Lewy bodies and Lewy neurites and is the defining histopathological hallmark of α-synucleinopathies. The three main α-synucleinopathy diseases include PD, Lewy Body Dementia (LBD), and multiple system atrophy (MSA) [29]. The most common α-synucleinopathy disease is PD [60]. Lewy neurites mostly have a course, thin and elongated appearance [61]. They are located in the cytoplasm and are greater in number than Lewy bodies, especially in the striatum and amygdala [62]. They are also heavily distributed in the dorsal vagal nucleus, the CA2/3 hippocampus region, and the nucleus basalis of Meynert [63]. Lewy neurites have been shown to impair axonal transport of autophagosomes and endosomes that contain Rab7 and TrkB receptors [62].

Lewy bodies are well-defined, spherical protein conglomerates composed of misfolded α-synuclein and other components. They are present in PD patients except for a handful of unique familial cases [64]. Lewy bodies are located in the neuronal cytoplasm and are found distributed across the brain stem, limbic areas, and neocortical brain regions [65,66]. Lewy body accumulation correlates aging, the severity of PD, and severity of dementia [67]. Likewise, α-synuclein in Lewy bodies is phosphorylated and nitrated, indicating oxidative stress is intrinsic to their formation [68-72]. Although widely recognized as contributing to neurodegeneration, there is some debate over whether Lewy bodies serve a protective role in the cell, if the process of forming the Lewy body promotes neurodegeneration, or if the Lewy body itself promotes neurodegeneration [33,73,74]. All three of these hypotheses are likely partially true. Furthermore, Lewy body composition has received renewed attention recently,

where the role of both α-synuclein and non- α-synuclein components are being reassessed. Some non- α-synuclein components of interest include ubiquitin, damaged organelles such as fragmented mitochondria, and lipids [75,76]. It is important to note that there is evidence to suggest that α-synuclein is not the most abundant constituent of Lewy bodies, contrary to the filament-centric dogma [75].

Misfolded proteins in neurodegenerative diseases have been shown to exist both intracellularly and extracellularly [77]. In PD, α-synuclein aggregations are seen earliest in the disease progression to be located in the olfactory bulb and the dorsal motor nucleus of the tenth cranial nerve [77,78]. The pathological α-synuclein then spreads rostrally through the brainstem, midbrain, forebrain, and eventually to the cortex [79]. α-synuclein has been shown to pass between neurons, from neurons to microglia, from neurons to astrocytes, between astrocytes, and across the blood-brain barrier (BBB) [80-83].

Microglial α-synucleinopathy in PD

Microglia are the CNS's resident immune macrophage that monitors for homeostatic threats and intervenes when necessary. Along with other glial populations, microglia are highly diverse based on their neuroanatomical location and functional plasticity, suggesting that they are influenced by local environment cues [84-87]. For example, microglia in healthy mouse basal ganglia regions had region-specific morphology, cell density, and count, lysosome content and distribution, membrane resting potentials, and transcriptomes [88,89]. Additionally, microglia experience altered intracellular α-synuclein levels based on their environment, such as in response to cytokines or cerebrospinal fluid (CSF) from PD patients [90,91].

Substantia nigra pars compacta (SNpc) microglia differ from microglia in the ventral tegmental area (VTA) [92]. Perhaps regional microglial differences might partly explain the PD-related loss of dopaminergic neurons in the SNpc, rather than in the VTA. The reasons for this regional selectivity of dopaminergic neuronal loss are not yet fully understood [92,93].

However, it seems like a reasonable hypothesis that microglial activation has a role to play. For example, mice overexpressing wild-type human α-synuclein throughout the CNS had increased levels of activated microglia and TNF-alpha in the striatum as early as one month old and then the substantia nigra as early as five months old, but not in other brain areas [94]. The region-specific activated microglial response to increased levels of α-synuclein persisted as long as monitoring took place, over 14 months [94]. In human PD patients, PET imaging and postmortem brain analysis showed regionally activated microglial cells in the midbrain, the frontal cortex, and the temporal cortexes [95,96]. Both 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and α-synuclein over-expression models of PD in monkeys also showed region-specific and long-term microglial activation in the SNpc [97,98]. Therefore, elevated α-synuclein levels cause microglia to become quickly and persistently activated, which leads to increased neuroinflammatory secretions from the microglia.

Among in vitro and in vivo models of PD, α-synuclein causes microglia to become rapidly activated, to migrate to the α-synuclein source, and then increases phagocytic and pro-inflammatory activity [99-102]. Extracellular α-synuclein is cleared through activated microglial engulfing and autophagy, mediated by TLR4-NF-kB signaling in a process recently discovered and coined as "synucleinphagy" [103]. However, microglial phagocytic activity is reduced with age [104]. The level of microglial activation is greater in the presence of α-synuclein mutants compared to wild-type α-synuclein protein, perhaps reflecting the severity of their respective associated pathologies [105,106]. Indeed, the PD-related α-synuclein A53T mutation has been shown to increase the production of microglial CXCL12 in cell culture and in mouse SNpc [107]. Postmortem brain tissue of PD patients have also shown a direct correlation between α-synuclein and CXCL12 levels [107].

Astrocytic α-Synucleinopathy in PD

Astrocytes are the most abundant cell type in the CNS, constituting an estimated 20% - 40% of the mammalian brain

and are about five times as prevalent as neurons [108-110]. Over twenty different structural and functional subpopulations of astrocytes exist in the adult human CNS [111,112]. Despite their heterogeneity, the overarching function of astrocytes is to provide support for neurons and to help ensure a homeostatic environment in the brain. Towards this end, astrocytes perform many functions. For example, they contribute to BBB maintenance, promote normal synaptic function, secrete neurotrophic molecules, modulate their microenvironment as necessary, and are involved with the regulation of neurogenesis, lipoprotein secretion, and cerebral blood flow [113]. Additionally, in experimental mouse models with either ablated or dysfunctional microglia, astrocytes became activated and phagocytic to help compensate for the microglial loss [114].

Even in their reactive state, astrocytes exhibit functional variability. From rest (A0), astrocytes can enter one of two polarized forms of activation based on their gene expression profile in response to stimuli: A1 or A2. In broad terms, A1 is neurotoxic and A2 is neuroprotective [113,115,116]. Astrocytes and microglia share a close relationship regarding reactivity and phagocytosis. Simply stated, reactive microglia give rise to astrocyte reactivity [115]. Indeed, the cytokines Il-1α, TNF-α, and C1q, which are released from reactive M1 microglia during chronic neurodegenerative conditions, are both necessary and sufficient for producing an A1 phenotype in astrocytes [115]. Blocking the ability of microglia to activate A1 astrocytes helps to preserve in vivo dopaminergic neuron viability [117]. A1 reactive astrocytes contribute to PD pathology by secreting a host of neurotoxic and pro-inflammatory factors that inhibit synaptogenesis and promote dopaminergic neuronal death [115]. Conversely, in response to traumatic or ischemic CNS injury, M2 microglia induce the genetic upregulation and downregulation of astrocytes to exert a neuroprotective A2 phenotype [118]. A2 astrocytes become reactive in response to microglial secreted IL-1β, IL-6, and nuclear factor IA (NFIA) as well as the silencing of miR-21 [119,120].

The A1 astrocyte phenotype has been observed in cultured astrocytes treated with α-synuclein aggregates [110,121].

Furthermore, cultured astrocytes readily take up secreted α-synuclein aggregates directly from neighboring co-cultured neurons to adopt an A1 phenotype [110]. Neuronally secreted α-synuclein binds to the toll-like receptors (TLRs) of astrocytes and promotes an increased neuroinflammatory response through activated TLR signaling [81,112,121,122]. The direct relationship between α-synuclein aggregates and reactive A1 astrocytes have also been observed in vivo, through a transgenic mouse model that expressed human α-synuclein [121]. Additionally, in postmortem PD patient brains, inclusions of α-synuclein aggregates have been found in astrocytes as well as in neurons [122,124-126]. When mutated α-synuclein is selectively expressed in mouse astrocytes, the astrocytes develop the A1 phenotype, neuroinflammation increases, microglia become activated and mice develop paralysis [127]. Despite the detrimental effects of reactive astrocytes induced by α-synuclein, astrocytes also contribute to the degradation and removal of mutated α-synuclein and are more efficient at it than neurons are [128].

Brain Cellular Senescence in PD

Introduction to Cellular Senescence

Three fates ultimately await very aged or damaged cells: senescence, apoptosis, or autophagy [129]. Autophagy (self-eating) is largely a homeostatic mechanism through lysosomal destruction of old or damaged cellular components, allowing for the recycling of cellular material. However, recent evidence also supports the view that autophagy plays an important role in mammalian cell death [130]. Apoptosis (self-killing) is a morphologically unique, genetically programmed cell death. It has important roles in development, aging, and atrophy, to maintain an appropriate level of cells [131]. It also has many critical roles in immune system function [132]. Cells can also enter a state of senescence (stable cell cycle arrest) as a homeostatic response to various stressors in order to mitigate the proliferation of damaged cells and to prevent neoplastic transformation [133]. Senescent cells are metabolically active, stable, and viable, unlike cells destined for death and recycling. The classical understanding is that only mitotically active cells in

the periphery can enter senescence by becoming arrested in the G_1 phase, unlike quiescent cells that arrest in G_0 [129,134]. However, post-mitotic cells in the central nervous system are now recognized as being able to undergo senescence [24]. There is some evidence of crosstalk and shared regulated pathways between these three cell fate states, but there is much that is currently not understood about their relationships to one another [135]. Also, there is some debate currently over the post-mitotic status of glia and CNS neurons.

Senescent cells accumulate during aging, both from an increased rate of production and a decreased clearance rate [136]. Although the accumulation of senescent cells is associated with increasing age, it is not age-dependent. Senescence is a dynamic and context-specific state that involves many biochemical pathways, such as the $p53/p21^{WAF1/CIP1}$ and $p16^{INK4A}$/pRB tumor suppression pathways [137,138]. Senescent cells that form in response to stress during earlier life stages, such as from oncogene activation, inflammation, or DNA damage, contribute to protective functions such as tumor suppression, wound healing, preventing the propagation of tissue damage, and embryogenesis [139,140]. In non-pathological states, senescent cells attract the immune system and are cleared [141].

There is a physiological threshold where senescent cells interfere with their own clearance, described by the recently proposed "immune threshold theory of senescent cell burden" [23]. According to this theory, once the saturation of senescent cells passes the senescent cell abundance threshold, the spread of cellular senescence outpaces the immune system's ability to clear them and cellular senescence becomes "self-amplifying" and contributes to age-associated diseases [23]. The age-associated nature of senescent cell accumulation and the threshold theory of senescent cell burden makes it a clear example of evolutionary antagonistic pleiotropy [142].
Senescent cells have characteristic phenotypes marked by irreversible and permanent cell cycle arrest, SASP, disruption of normal mitochondrial form and function, metabolic changes, genomic DNA damage, telomere attrition, altered epigenetics, impaired DNA repair mechanisms, altered proteostasis,

increased reactive oxygen species (ROS) production and increased ROS-mediated damage [143]. The phenotypical changes that senescent cells undergo allow the cell to influence its immediate environment through SASP as well as to ensure cell cycle arrest [144]. The hypersecretory phenotype SASP is a key hallmark of senescent cells. The composition of SASP includes pro-inflammatory cytokines such as IL-6 and IL-8 as well as insulin-like growth factors IGFBP3, IGFBP4, and IGFB7, which have pro-senescence and tumor-suppressant properties [145,146]. In addition to cytokines and growth factors, SASP also includes chemokines, matrix-metalloproteinases, and many other constitutes [147]. The exact composition of SASP is dynamic and heterogeneous depending on the senescent cell type, driver of senescence, and cellular context [144,148,149].

SASP works to reinforce the cellular senescence of its source through the autocrine senescence process as well as spreading senescence to non-senescent neighboring cells via paracrine senescence [150,151]. SASP can be activated in various ways, but it has been argued that DNA damage is the main upstream driver [137]. The NF-κB and JAK2/STAT1 pathways are the main regulators of SASP [152]. Therefore, SASP expression is influenced by pathways that modulate the NF-κB and JAK2/STAT1 pathways, such as the mammalian target of rapamycin (mTOR) pathway, mitogen-activated protein kinase (MAPK) signaling, the phosphoinositide 3 kinase (PI3K) pathway, the DNA damage response (DDR), and GATA4/p62-mediated autophagy [144,146].

Microglial Senescence in PD

Senescent microglia differ from activated microglia. Microglia undergo non-pathologic changes related to advanced age that brings them to a senescent state, sometimes synonymously referred to as dystrophy, although dystrophy typically only refers to the morphological changes of senescent microglia [153-157]. Dramatic morphological dystrophy of senescent microglia is not seen in the brains of mice but is observed in humans [157]. Senescent microglia undergo distinct age-related morphological, functional, and distribution changes [92,157,158]. For example,

senescent microglia experience swelled spherical somas, a fragmented and beaded cytoplasm, and reduced ramification (very shortened processes, a reduction in the number of processes, and a reduction in the number of branches per process) [92,158]. Senescent microglia also experience a change in their uniform distribution resulting in increased location-specific density [159]. The total number also increases with age, although this seems debatable [159-161].

Functionally, senescent microglia resemble a mild activation state [157,162,163]. For example, there is an increased proportion of senescent microglia in the aged brain that experience spontaneous Ca^{2+} transients compared to middle-aged or young brains in mice [164]. Neurodegenerative insult still elicits an attempt by aged microglia to boost their heightened baseline activation levels. For example, aged mouse models of Alzheimer's disease experience a higher degree of intracellular Ca^{2+} transients in senescent microglia than what is seen due to aging alone [165]. Additionally, senescent microglia have a reduced rate of migration to injured areas and a reduced rate of process movement towards injury [158,166]. Furthermore, aged mice have impaired microglial phagocytic ability [161]. As the proportion of senescent microglia accumulates with age or age-related disease, they eventually outnumber healthy microglia and produce an enhanced neuroinflammatory response to injury, compared to young brains [167-169]. Together, these observations support the notion of impaired function of senescent microglia in aged brains.

As the brain ages, it experiences chronic low-level oxidative stress and subsequent inflammation [157]. Aging drives the switch from neuroprotective microglia to senescent microglia, as well as an increased concentration of senescent microglia in the SNpc [153]. As part of the age-related inflammatory milieu of the CNS, senescent microglia chronically secrete pro-inflammatory cytokines such as IL-6, IL-8, IL-1β, and TNF-α [159,166,170]. They also release a reduced amount of anti-inflammation cytokines and increased levels of ROS, which are Nox-2 dependent [153,171]. The pro-inflammatory secretions of senescent microglia are akin to the pro-inflammatory secretions

of activated microglia. However, consistent with a senescent state, senescent microglia also have genomic and mitochondrial DNA damage as well as telomere shortening [88,172]. Senescent microglia also have upregulated expression of senescent markers such as Bcl-2, senescence-associated β-galactosidase (SA-β-gal), $p16^{INK4a}$, $p21^{WAF1/CIP}$, lipofuscin, and H2AX[pS139] [161].

The senescent microglial response to injury is delayed and prolonged, which helps to shape the chronic and slowly progressive nature of PD [167,173]. The significance of an aged CNS with senescent microglia in PD pathology is demonstrated clearly in mouse models. For example, a low dose of the pesticide rotenone reduced the number of SNpc dopaminergic neurons by up to 30% in old rats but was benign when given to young rats [174]. Similarly, old mice with age-related senescent microglia experienced a markedly more severe reduction of dopaminergic neuron concentration in response to MPTP injections than neonatal mice [175]. Despite the value of toxin rodent models in the study of PD, it is noted that there are some limitations in their applicability [176].

PD has been epidemiologically linked to iron exposure [153]. Analysis of SNpc tissue from postmortem PD patients also reveal an elevated iron concentration compared to controls [153]. Despite not being the main cell type to store iron in the brain, senescent microglia have increased levels of ferritin, which increases their internal exposure to iron-related oxidative stress [153,162,177]. Oxidative stress in microglia, whether due to heightened iron levels or catabolizing degenerative neurons that contain iron-rich neuromelanin, prompts senescent microglial secrete pro-inflammatory factors. Therefore, in addition to aging and α-synucleinopathy, intracellular iron contributes to the senescent secretome.

There are morphological and physiological differences between male and female microglia. The sex-related differences in microglia correspond to the sex differences in PD risk. Men have between 1.5x and 2x the risk for developing PD than females [178,179]. Furthermore, estrogens are neuroprotective against PD [180]. The sex differences in microglia are thought to be the

result of estrogen priming [178]. For example, there is evidence that the female 6-OHDA mouse model of PD has higher levels of estrogens than males, which prompts their activated microglia to polarize towards the neuroprotective M2 phenotype rather than the male-dominant pro-inflammatory M1 phenotype [181]. The sex differences in brain inflammation response, PD risk, and microglial physiology due to estrogen activity has also been confirmed in humans [182-184].

Astrocytic Senescence in PD

Senescence and reactivity are distinct astrocytic cell fates, yet they share some common features. A recent transcriptomic study revealed a wide range of senescent-related markers upregulated in senescent astrocytes such as SA-β-gal, IL6, IL8, IL1A, IL1B, *CDKN1A*, the p53/p21^{WAF1} and p16^{INK4A}/pRB pathways, *CYR61*, *CCND1*, *IGFNP5*, and *IGFBP2* [185]. Senescent astrocytes also experience an upregulation of high-mobility group B (HMGB) proteins, increased production of vimentin and glial fibrillary acidic protein (GFAP), in addition to a reduced expression of neurotrophic factors and nuclear lamina protein laminB1 [186]. Some of the upregulated inflammatory marker genes are shared between reactive and senescent astrocytes. For example, astrocytes in both states experience upregulated pro-inflammatory cytokines, chemokines, proteases, and growth factors [149,185,187]. The morphology of senescent astrocytes change to become flattened, enlarged, and have vacuolized lysosomes [187,188].

Normal aging gives rise to astrocytic senescence-inducing factors such as damaged DNA and shortened telomeres [189,190]. Senescent astrocyte accumulation also occurs in neurodegenerative diseases [191]. Compared to control tissue, the SNpc tissue from five postmortem PD patients exhibited a large increase in the levels of p16^{INK4a} and the SASP components MMP-3, IL-6, IL-1α, and IL-8, as well as reduced nuclear level of lamin B1 [192,193]. Interestingly, the drop in nuclear lamin B1 levels was only observed in astrocytes, whereas nuclear lamin B1 levels remained unchanged in the astrocyte-neighboring tissues between the PD SNpc and control tissues

[192]. These results suggest that astrocytes have a uniquely elevated susceptible to becoming senescent in PD. Furthermore, multiple studies have shown that cultured human and rodent astrocytes are more susceptible to toxin-induced senescence than fibroblasts [188,192]. However, one study showed that neurons in long-term cultures of primary rat cortical cells became senescent before neuroglia did [194]. It is thought that this aberrant result was due to dysfunctional age-associated autophagy [194]. Senescent astrocytes also have decreased expression of glutamate transporters, and therefore, promote glutamate toxicity and subsequent death of surrounding neurons [191].

Paraquat has been a widely used toxic herbicide and has a chemical structure similar to the dopaminergic neurotoxin MPTP [195]. Whether chronic occupational exposure to paraquat is causal for PD in humans is debated. Although the connection between paraquat and PD in humans has seemed strong in the past, the more current perspective considers the correlation to be weak [196]. Regardless, paraquat has been successfully used for animal models of PD, replicating important hallmarks of the disease such as increased α-synuclein levels and α-synuclein aggregations in SNpc neurons, leading to dopaminergic neuron loss and movement impairment [197]. hiPSCs that have been differentiated into astrocytes and exposed to paraquat cease to proliferate and have increased levels of SA-β-gal, $p16^{INK4a}$, and IL-6, indicating a senescent state [192]. The hiPSC derived astrocytes also had an increased number of 53BP1 foci due to the upregulation of DNA damage signaling, which stimulates SASP expression [192]. Other environmentally toxic chemicals that have been linked to PD, like the pesticide rotenone and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), have also been shown to cause premature senescence in human astrocytes in a dose-dependent manner [185,198,199].

The oxidative stress and subsequent inflammation associated with neurodegenerative diseases, non-pathological advanced age, and environmental toxin exposure drive elevated senescent astrocyte levels [188,200]. For example, the hormone angiotensin II has been implicated in producing intracellular free

radicals, increasing oxidative stress, promoting mitochondrial dysfunction, and accelerating inflammation related to aging [201]. Angiotensin II has also been shown to cause senescence in cultured human astrocytes in a concentration-dependent manner by producing superoxide oxidative stress [202]. The transcriptome of fetal human astrocytes that have been made senescent by transient oxidative stress exposure have been characterized. Unsurprisingly, genes associated with neural development and differentiation were downregulated as well as some genes related to injury response, whereas pro-inflammatory genes were upregulated [203].

Interactions between α-Synucleinopathy and Neuroglial Senescence in PD

A Self-Amplifying and Vicious Cycle

The physiological connections between senescent neuroglia and the development of neuronal α-synucleinopathy are feed-forward oxidative stress and inflammation cascades. The brain is a highly metabolically active organ and consumes about 20% of the basal oxygen in humans [204]. Free radicals are abundant in the brain and are necessary for the delicate redox signaling that is critical for many CNS functions [204-206]. However, when the oxidative balance favors cellular stress, cellular damage results. According to the free radical theory of aging, a consequence of aerobic metabolism over the lifespan is oxidative damage that results in the aging process [207].

CNS oxidative stress has been shown to induce neuroglial senescence. Specific oxidative stress effects on neuroglia that lead to a senescent state include morphological changes, disrupted mitochondrial function, altered cellular signaling, altered cellular metabolism, damaged DNA, shortened telomeres, altered chromatin structure, altered proteostasis, and impaired DDR [143,208,209]. The pro-inflammatory SASP of senescent astrocytes and microglia contribute to the low-grade chronic inflammation that is associated with CNS aging [143,157,210,211]. CNS inflammation contributes to the α-synuclein toxicity that drives α-synucleinopathy in PD. Neuroinflammation is increasingly being recognized as one of

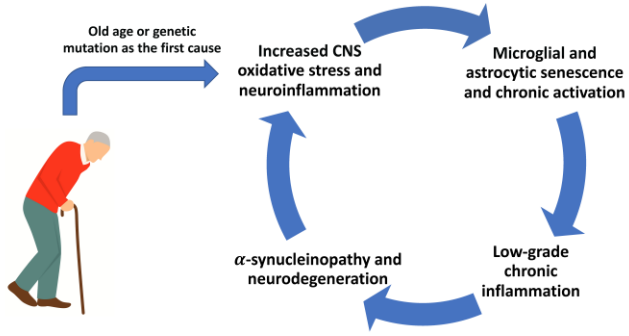
the major contributors to PD pathology rather than just a by-product of the disease process itself [176,212].

Neuroinflammation in PD involves both the innate immune system and the adaptive immune system since PD-related BBB compromise facilitates the entrance of peripheral immune cells into the CNS [213,214]. The two Nod-like receptor (NLR) proteins, NLRP3 and NLRC4, are part of the innate immune system, are inflammasome activators in microglia and astrocytes, and activate caspase-1 [215]. NLRP3 activation in microglia by ROS leads to the release of the pro-inflammatory cytokines IL-1β and IL-18 [85]. ROS can also activate NLRC4 in astrocytes, prompting the release of IL-1β and IL-18 as well [216].

Concerning PD pathology, activated caspase-1 cleaves α-synuclein into a truncated form that is highly prone to forming the insoluble aggregates characteristic of Lewy bodies [217]. There is robust *in vitro* evidence that inflammasome-activated, caspase-1-induced α-synucleinopathy is cytotoxic [217,218]. Caspase-1 has also been shown to truncate α-synuclein at the C-terminal end in the proteolipid protein α-synuclein (PLP-SYN) transgenic mouse model of MSA, which promoted α-synuclein aggregation, motor deficits, and a reduction in tyrosine hydroxylase-positive neurons in the substantia nigra [219]. Furthermore, the capase-1 inhibitor VX-765 is neuroprotective in the PLP-SYN mouse model [219]. Caspase-like truncation of the C-terminal end of α-synuclein has been discovered and characterized in other PD models of transgenic mice, cell culture, and recombinant adeno-associated virus injected rats [220,222]. Truncated α-synuclein as well as full-length α-synuclein have been widely observed in post-mortem brains from PD patients and patients with Dementia with Lewy bodies [39]. Therefore, when microglia and astrocytes are exposed to age-related chronic oxidative stress, they progress to releasing the pro-inflammatory SASP and convert WT α-synuclein into a PD-related toxic species.

Senescent neuroglia prime neurons for neurodegeneration and contributes to the early pathology. For example, when healthy neurons are co-cultured with senescent neuroglia, they

experience a reduction in function, synapse maturation, synaptic plasticity, synaptic vesicle size, and disrupted neuronal homeostasis [186,191,223,224]. The factors involved in senescent neuroglial mediated neuronal detriment include the pro-inflammatory secretion, reduced neurotrophic factor secretion, reduced glutathione secretion, and the reduced ability to clear extracellular α-synuclein [163,225]. Furthermore, it has been shown that removing senescent neuroglia from models of neurodegeneration mitigates reactive gliosis and neuronal death while preserving normal organismal physiology [192,226] opagating cycle of oxidative stress, neuroinflammation, neuroglial senescence, neuroglial activation, and neuronal death. The injured, degenerative dopaminergic neurons in PD release insoluble α-synuclein fibrils, ATP, MMP-3, and neuromelanin into the extracellular space [227]. In an effort to maintain brain homeostasis, microglia and astrocytes are prompted to take up the cellular debris from degenerating neurons and initiate the subsequent inflammatory and oxidative stress responses. Although these actions of neuroglia are necessary and beneficial in a non-pathologic state, when they can no longer combat the tide of degeneration, the cycle becomes self-amplifying and destructive. Figure 3 presents the interactions between α-synucleinopathy and neuroglial senescence in PD.



The Effects of Neuroglial Uptake of α-Synuclein and Activation by α-Synuclein

Neurons secrete α-synuclein into the extracellular space through an exosomal and calcium-dependent manner [228]. The rate of α-synuclein secretion from neurons and neuroblastomas into the extracellular space, as well as the concentration of secreted insoluble α-synuclein aggregates, increases under cellular stress-induced protein misfolding and damage [229]. The damaged, aberrant α-synuclein are secreted into the extracellular space under stress through exocytosis rather than in exosomes [229]. Extracellular wild-type and mutant α-synuclein can be taken up by neurons or glia via endocytosis and transmitted between glia and neurons, even in aggregated form, leading to the spread of Lewy bodies [230,231].

Extracellular non-mutated α-synuclein oligomers elicit an inflammation response from microglia via paracrine activation of Toll-like receptor 2 (TLR2) [230,232]. Moreover, extracellular α-synuclein can act as damaged-associated molecular patterns (DAMPs) to activate other microglial receptors and intracellular pathways, such as the Fc gamma receptor IIB (FcγRIIB) and the NF-κB pathway [85]. The activation of these receptors and pathways lead to reduced microglial phagocytosis, an upregulated inflammation response, α-synuclein nitration and increased release of ROS [85,99,102].

A robust debate over whether activated microglia are harmful or beneficial spans back more than twenty years [233]. In PD, chronically activated microglia increase neuronal susceptibility for neurodegeneration. Permanently senescent microglia clearly differ from the transiently activated microglia. Microglia become transiently activated in response to brain injury and upregulate both pro-inflammatory and anti-inflammatory genes simultaneously [234]. However, the chronically activated microglia that are commonly observed in neurodegeneration seem to significantly differ from senescent microglia in semantics only [235,236]. Naturally, there are slight differences between senescent and chronically activated microglia, but they seem to be functionally very similar in PD. We hypothesize that

the senescent, chronically activated, or transiently activated fates reflect the heterogeneity of microglial populations and the effects of individual microglial microenvironments [237,238]. Operating under this hypothesis, it is possible that some senescent microglia adopt a chronically activated phenotype due to an early neurodegenerative milieu and contribute to the further development of the neurodegeneration in a chronic manner [237].

Chronically activated microglia contribute to the early development of PD in response to interacting with α-synuclein and remain chronically activated as long as α-synuclein is present. For example, an in vivo mouse model that overexpressed full-length, wild-type, human α-synuclein under the murine Thy-1 promoter experienced lifelong activation of microglia [239]. Activated microglia first appeared in the striata at 1 month old and in the SNpc at about 5 months old, before neuronal damage was observed [239]. People with idiopathic rapid-eye-movement sleep behavior (IRBD) serve as an interesting population for studying the preclinical hallmarks of PD. Nine out of ten people with IRBD receive a PD diagnosis within fourteen years of receiving an IRBD diagnosis [240]. The pathology is strikingly similar between the two diseases. For example, striatal ^{18}F-DOPA is reduced in 90% of IRBD patients, coupled with unilateral microglia activation in the SNpc, consistent with early PD [241]. These observations correlate with the notion that inflammation due to chronically activated microglia facilitates the change from IRBP to PD over time. Indeed, postmortem PD brains have substantial quantities of pro-inflammatory microglia and astrocytes in the same areas affected by α-synucleinopathy [176,192,193,242].

Extracellular α-synuclein endocytosed by astrocytes cause the release of proinflammatory and neuroinhibitory secretions, such as GFAP, cytokines, chemokines, and chondroitin sulphate proteoglycan [243]. However, there is some evidence that α-synuclein uptake by astrocytes follows α-synuclein binding to astrocyte receptors to stimulate proinflammatory secretions [243]. Endocytosed α-synuclein in astrocytes causes disrupted Ca^{2+} and mitochondrial homeostasis, oxidative stress, and

elevated levels of glutathione peroxidase [99]. Astrocytes can harbor insoluble α-synuclein aggregates [121]. The uptake of α-synuclein aggregates by astrocytes is initially a protection mechanism that aims to clear the toxic protein [122]. However, the pathological inclusion can cause lysosomal dysfunction, can remain in astrocytes, and therefore, accumulate and cause cellular damage [122].

Astrocytes have a unique susceptibility to becoming senescent in response to cellular stress. In response to oxidative stress, astrocytes become senescent before fibroblasts, neurons, and possibly microglia [188,192]. In postmortem PD SNpc tissue, the only monitored cell type to have a reduction in nuclear lamin B1 compared to control SNpc tissue were astrocytes [192]. Reduced lamin B1 levels are a long standing biomarker of cellular senescence [193]. However, microglia are the first line of defense in response to injury and oxidative stress. They exhibit fast migration to the injured area in order to initiate phagocytosis and quickly become activated. Soon afterwards, microglia recruit astrocytes to become activated, release pro-inflammatory factors and promote glutamate-induced excitotoxicity [115,244-246]. If this same pattern is seen in neurodegeneration-related chronic neuroglial activation, then microglia would become "senescent" first. Even if astrocytes become senescent before their cellular neighbors do, the bystander effect ensures the spread of senescence to other cell types [247].

The Effects of Neuroglial Exposure to Preformed α-Synuclein Fibrils

There are three morphological species of α-synuclein. From smallest to largest, they are monomers, oligomers, and fibrils. The major pathological components of Lewy Bodies are α-synuclein fibrils. Artificially synthesized "preformed α-synuclein fibrils" (PFFs) can be injected into animal models of PD to study the spatiotemporal dynamics of α-synuclein movement, inflammation, oxidative stress and neurodegeneration. In response to the presence of α-synuclein fibrils, neuroglia generate superoxide (O2-), ROS, and cytotoxic

factors [227]. PFFs also exert a strong response in dopaminergic neurons. For example, dopaminergic neurons that are treated with synthesized PFFs experience elevated levels of serine 129 phosphorylated α-synuclein, increased α-synuclein aggregation, reduced levels of presynaptic protein, axonal transport protein disruption, and reduced dopaminergic survival [248]. These results are attributed to PFF-induced mitochondrial disfunction, increased O2- production, increased nitric oxide (NO) production, levels of protein nitration, and inflammation [248].

In a recent study, PFFs were injected into the striata of healthy, young adult, wild-type (C57BL/6) male mice, which initially caused an inflammation response in both astrocytes and microglia [249]. The inflammatory state peaked at seven days post-injection (dpi). Aggregates of α-synuclein then increased in concentration and propagation after fourteen dpi and peaked between thirty and ninety dpi [249]. Finally, striatal dopaminergic neuron loss and motor disfunction were observed [249]. Similar results were seen in male Fischer 344 rats that received unilateral intrastriatal injections of PFFs [250]. Microglial activation and associated inflammation were the initial responses to PFF injection in the rats [250]. Microglial activation in response to the PFF lasted for at least the three months before SNpc neuronal degeneration occurred and persisted throughout the degenerative process, indicating that the microglia were chronically activated [250].

In another recent study described here, PFF gave rise to activated neuroglia, senescent neuroglia and neuronal death [251]. MPP^+ or PFF treatment of cultured dopaminergic rat N27 cells caused the cells to express senescence markers, such as reduced levels of Lamin B1 and HMGB1 and increased levels of p16 and p21. PFF treatment of cultured primary astrocytes and microglia from C57BL/6 wild-type mice also lead to senescence, as evidenced by decreased Lamnin B1, HMGB1, AT-rich sequence-binding protein 1 (SATB1), and p16 levels, but elevated p21. Interestingly, there was simultaneous production of senescent and reactive astroglia in response to primary culture PFF treatment. This observation might reflect the different subpopulations of astrocytes [111]. Mice that received brain PFF

injections through cannula demonstrated the same changes in senescent markers. For example, the ventral midbrain and SNpc of these mice experienced reduced levels of Lamnin B1, HMGB1, and p16 levels and increased levels of p21. Increased levels of GFAP and Iba-1 were also seen, indicative of reactive astroglia and microglia respectively. Moreover, there was evidence of neuronal death by reduced levels of β-III-tubulin. Finally, SNpc tissue from postmortem PD patients confirmed the involvement of cellular senescence by western blot analysis. Lamnin B1, HMGB1, and SATB1 were reduced, p21 levels were increased and p16 levels remained unchanged in postmortem PD brains compared to control midbrain tissues. The experimental results from Verma et al., [251] highlights how pathologic α-synuclein instigates simultaneous neuroglial senescence and neuroglial activation that eventually lead to PD-relevant neuronal death.

Senolytics as a Therapeutic Avenue for PD

Senescent cells contribute to a variety of age-related diseases. Conversely, their removal mitigates their associated pathological effects and increases the healthspan. Reduced activity of the SATB1 protein in dopaminergic neurons has recently been identified as a risk factor for PD [252-255]. Furthermore, genetic knockout of *Satb1* leads to cellular senescence and increased expression of p21 and CDKN1A in human embryonic stem cells that have differentiated into dopaminergic neurons [256]. Riessland et al. [256] also showed this phenomenon in the midbrains of mice by using a stereotactic adeno-associated virus 1 injection expressing shRNA (AAV1-shRNA) to downregulate *Satb1*, which subsequently increased p21 expression and neuronal senescence. Eventually, treatment with AAV1-shRNA eliminated tyrosine hydroxylase expressing neurons, reduced the number of mitochondria, upregulated *Cdkn1a*, and prompted an immune response [256]. Additionally, postmortem SNpc tissue from PD patients have elevated p21 expression and decreased regulatory function of SATB1 [252,255,256]. Inhibition of p21 in SATB1 knockout human dopaminergic neurons via the p21 inhibitor UC2288 significantly reduced the effects of senescence without producing proliferation [256]. Furthermore, treatment of SATB1 knockout human dopaminergic neurons with CDKN1A

short hairpin RNA (shRNA) dramatically reduced p21 levels and other senescence hallmarks [256]. Additionally, UC2288 has recently been shown to reduce senescence markers such as oxidative stress and inflammation in the MPTP mouse model of PD [257]. Therefore, UC2288 might be a viable anti-senescent agent for PD.

Astragaloside IV (AS-IV) is the active pharmacological agent derived from the herbal plant Astragalus membranaceus. AS-IV has a long history in Chinese herbal medicine due to its many beneficial properties, such as being a powerful antioxidant, antifibrotic and anti-inflammatory agent [258]. AS-IV is neuroprotective in primary dopaminergic nigral cell culture exposed to 6-hydroxydopamine [259]. Mice treated with chronic MPTP and probenecid injections experience a significant loss of dopaminergic neurons in the SNpc and suffer from loss of muscle strength and balance [206]. However, when receiving co-treatment with AS-IV, dopaminergic neurons and motor deficits in MPTP and probenecid treated mice have significant protection without altering MPTP metabolism [260]. An important finding from the Xia et al. [260] study was that AS-IV treatment reduced the SNpc concentration of senescent astrocytes in the MPTP mouse model and improved many markers of cellular senescence, such as elevated p16 levels and reduced levels of lamin B1 in the cellular nucleus. Furthermore, natural age-related senescence and premature senescence due to MPP+ treatment in primary astrocyte culture from mice were shown to be inhibited by the AS-IV treatment [260]. AS-IV was shown to exert its anti-senescent effect through promoting mitophagy and its antioxidant properties [260].

It has recently been shown that astrocyte and microglia senescence in PD can be mitigated by senolytic treatment with the serum and glucocorticoid related kinase 1 (SGK1) inhibitor GSK-650394 [261]. NF-kB transcription factors are responsible for transcribing pro-inflammatory genes, including those for cytokines and chemokines [262]. Through phosphorylation, SGK1 activates NF-kB pathways and promotes inflammatory responses [263]. GSK-650394 reduces cytokine levels and SGK1 overexpression boosted cytokine levels in

cultured mice astrocytes and microglia from the cortex and ventral midbrain [261]. Furthermore, Nurr1 and Foxa2 downregulate *Sgk1* in the mouse cultured glia, as shown in microarray and RNA-seq data [261]. Seven out of the top ten genes that were downregulated by GSK-650394 had immune-related ontologies [261]. Therefore, the anti-inflammatory effects of Nurr1 and Foxa2 in glia are due to inhibitory action on *Sgk1*.
It has also been shown that SGK1 inhibition suppresses inflammation pathways associated with the NLRP3 inflammasome and CGAS-STING, upregulates glutamate clearance from glia, and prevents glial mitochondrial damage [261]. Finally, SGK1 inhibition reduced glial senescent markers such as SA-β-gal, downregulated genes associated with SASP, reduced pro-senescent protein levels, reduced reactive oxygen species production, and downregulated pro-oxidant genes [261]. Importantly, mouse midbrain dopaminergic neurons that overexpressed human α-synuclein were co-cultured with mouse ventral midbrain astrocytes and microglia. These cultures were treated with PFFs. Culture treatment with GSK-650394 or SGK1 knockdown in the glia reduced α-synuclein pathology in neurons including α-synuclein neuron-to-neuron transfer, provided they were co-cultured with the ventral midbrain glia [261]. Furthermore, SGK1 inhibition in mouse ventral midbrain astrocytes and microglia co-cultured with mouse midbrain dopaminergic neurons protected the neurons from toxic insult from H_2O_2. Finally, SGK1 genetic silencing or GSK-650394-mediated inhibition in the MPTP mouse model of PD protected against behavioral deficits, midbrain dopaminergic neurons loss, and suppressed SNpc inflammation and senescence [261].

B-cell lymphoma-extra large (Bcl-xL) is a member of the Bcl-2 protein family and resides in mitochondrial membranes. Bcl-xL has anti-apoptotic properties mediated through its inhibition of mitochondrial cytochrome c release [264]. Furthermore, Bcl-xL also has pro-senescent properties. For example, it is hypothesized that damaged cells otherwise destined for apoptosis can instead become senescent through the overexpression of Bcl-xL [265]. Bcl-xL also enhances mitochondrial metabolism and increases the efficiency of ATP synthesis, both of which are

necessary to metabolically support the increased SASP production of senescent cells [265,266].

In postmortem brain samples from PD patients, it was shown that Bcl-xL expression in mesencephalon dopaminergic neurons was close to twice as high as in the controls [267]. Interestingly, Bcl-xL is likely involved in sporadic PD through pro-senescence and anti-Parkin activity. Under normal conditions, the PINK1 protein cooperates with the protein Parkin to translocate to polarized mitochondria and induce mitophagy. However, mutated E3 ubiquitin ligase Parkin and pathological mitochondrial bioenergetics are implicated in autosomal recessive familial PD [268]. It has been shown that Bcl-xL antagonizes the ability of PINK1 and Parkin to stimulate mitophagy [265]. Disrupted midbrain mitophagy is a foundational pathological feature common in both PD patients and PD animal models [269]. Therefore, it seems likely that Bcl-xL inhibitors, such as A1331852 and A1155463, could be effective therapeutic agents in PD through promoting mitophagy [270].

The relationship between PD pathology and Bcl-xL is complicated. Like cellular senescence, Bcl-xL seems to have the capacity in PD for neuroprotection, as well as exacerbating pathology. For example, SH-SY5Y cells transfected with a dopamine transporter were resistant to MPP^+ when treated with Bcl-xL [271]. SH-SY5Y cells overexpressing Bcl-xL were also resistant to 6-hydroxydopamine induced death [272]. Additionally, SH-SY5Y cells overexpressing Bcl-xL preserved mitochondrial dynamics through anti-oxidative stress mechanisms when LRRK2 was pharmacologically inhibited by GSK2578215A [273]. Furthermore, Bcl-xL treatment was shown to be neuroprotective in the MPTP mouse model of PD [271]. Finally, Bcl-xL is necessary for CNS synapse formation, synaptic vesicle membrane dynamics, and neurite outgrowth, all of which become disrupted during neurodegeneration [274,275]. Although these results were only demonstrated in cell culture and imperfect mouse models of PD, they do raise some hesitancy for pursuing senolytic Bcl-xL antagonists as a therapeutic avenue.

Despite the potential dual role of Bcl-xL in PD, perhaps the different effects can be parsed and capitalized on. The Bcl-xL protein can be cleaved at its N-terminus by caspase-dependent mechanisms to produce ΔN-Bcl-xL fragments. Bcl-xL fragmentation is increased during glutamate-induced neuroexitotoxicity, which commonly occurs in many neurodegenerative diseases, including PD [244,276]. Accumulation of ΔN-Bcl-xL fragments induces mitochondrial injury, such as elevated membrane conductance and increased cytochrome c release, eventually leading to neuronal death [276]. The senolytic ABT-737 binds to both Bcl-xL and ΔN-Bcl-xL, prevents ΔN-Bcl-xL from damaging mitochondria, and prevents the Bcl-xL from forming ΔN-Bcl-xL fragments [276]. Furthermore, it has been shown that the effects of Bcl-xL senolytics are concentration-dependent. For example, high concentrations of ABT-737 (1 μM) and WEHI-539 (5 μM) exacerbated neurotoxicity from glutamate, disrupts mitochondria membrane potential, and reduces the cellular concentration of ATP [277]. Conversely, a low concentration of ABT-737 (10 ηM) and WEHI-539 (10 ηM) was neuroprotective against glutamate-induced cell death through protecting mitochondrial membrane potential and preserving ATP loss [277]. Together, the evidence seems to suggest that Bcl-xL still holds promise as a target in the anti-senescence treatment of PD, but the concentration of Bcl-xL specific senolytics and Bcl-xL fragmentation potential needs to be taken into consideration. Since Bcl-xL fragmentation occurs in response to glutamate neurotoxicity, perhaps Bcl-xL specific senolytics would have more effect before disease onset.

Anti-senescent or senescent cell removal strategies seem like a possible new avenue of pharmacological therapy for PD patients. However, the majority of evidence is currently limited and restricted to cell and animal models. Indeed, the majority of therapeutic studies of senolytics are pre-clinical. However, the first open-label, single-arm clinical trial of senolytics in human patients was published in 2019 [278,279]. This study showed that short-term (3 weeks) treatment of senolytic cells in patients with idiopathic pulmonary fibrosis with dasatinib and quercetin (D + Q) improved symptoms and function [278,279]. Since

then, D + Q has also been shown to be effective at decreasing senescent cells in diabetic kidney disease patients [280]. There has been a rapid increase in the number and scope of the clinical trials centered on senolytics just during this past year [278,281,282]. Concurrently, there has also been a rapid rise in pharmaceutical companies and capitalist investments focused exclusively on developing senolytics over the last handful of years [283].

There are fourteen clinical studies currently listed at ClinicalTrials.gov that result from searching "senolytic" in the "other" search field. Four trials target osteoarthritis, four are focused on mitigating COVID-19, and the rest center on femoroacetabular impingement, frailty in adult survivors of childhood cancer, chronic kidney disease, and improving the skeletal health of healthy older adults. In a variety of combinations and dosings, the senolytics D, Q, and fisetin are included as drug interventions in all of these clinical trials. One of the osteoarthritis trials includes fisetin and also the antihypertensive drug losartan. Another current osteoarthritis trial includes Q, fisetin, and also glycyrrhizin as interventions. Glycyrrhizin has anti-inflammatory and antiviral properties. Previous or planned senolytic focused clinical trials have employed their use in the treatment of hyperoxia-induced reactive airway disease, insulin resistance, diabetes, pre-eclampsia, fatty liver disease, obesity, macular degeneration, and diabetic chronic kidney disease [278,281].

Out of the fourteen results, only two trials focus on neurodegeneration: a pilot and phase II trial of the SToMP-AD study (Senolytic Therapy to Modulate the Progression of Alzheimer's disease). The pilot study (ClinicalTrials.gov Identifier: NCT04063124) focuses on the use of D + Q for five patients with early-stage Alzheimer's disease over a 12-week period [284]. The Phase II SToMP-AD study is currently recruiting and plans to include both patients with Alzheimer's disease and Mild Cognitive Impairment (ClinicalTrials.gov Identifier: NCT04685590). It is fully expected that effective senolytic-based therapy for PD patients and their families will be a reality in the not-too-distant future.

Table 1: Anti-senescent drugs that have therapeutic potential relevant for Parkinson's disease.

Senolytic	Evidence	Citation
UC2288	Reduces neuronal senescence in MPTP mouse model and primary cell culture through inhibiting p21, oxidative stress and inflammation	[256]
Astragaloside IV	Removed senescent astrocytes in MPTP mouse model and MPP+ cell culture through promoting mitophagy and antioxidant properties	[260]
GSK-650394	Inhibits astrocyte and microglia SGK1 mediated neuronal senescence and inflammation	[261]
ABT-737 and WEHI-539	Low concentrations inhibit Bcl-xL fragmentation and ΔN-Bcl-xL mediated neuronal damage	[277]

Conclusion

PD is the most common movement disorder and the second most common neurodegenerative disorder. However, the complex pathology is not yet fully understood nor is there a cure available. The study of PD has largely focused on neurons since the disease is marked by progressive neurodegeneration. PD research has also largely centered on aggregated α-synuclein since they are the key molecular hallmark of the disease. However, neuroglia account for a large portion of the brain and are responsible for a myriad of critical functions in the CNS. The roles of neuroglia in neurodegenerative diseases are underappreciated. Targeting senescent neuroglia in PD is an exciting possible therapeutic avenue. Clinical trials of anti-senescent drugs have very recently started to get underway and hold much promise.

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