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# Are There Roles for Brain Cell Senescence in Aging and Neurodegenerative Disorders?

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## Abstract

The term cellular senescence was introduced more than five decades ago to describe the state of growth arrest observed in aging cells. Since this initial discovery, the phenotypes associated with cellular senescence have expanded beyond growth arrest to include alterations in cellular metabolism, secreted cytokines, epigenetic regulation and protein expression. Recently, senescence has been shown to play an important role *in vivo* not only in relation to aging, but also during embryonic development. Thus, cellular senescence serves different purposes and comprises a wide range of distinct phenotypes across multiple cell types. Whether all cell types, including post-mitotic neurons, are capable of entering into a senescent state remains unclear. In this review we examine recent data that suggest that cellular senescence plays a role in brain aging and, notably, may not be limited to glia but also neurons. We suggest that there is a high level of similarity between some of the pathological changes that occur in the brain in Alzheimer's and Parkinson's diseases and those phenotypes observed in cellular senescence, leading us to propose that neurons and glia can exhibit hallmarks of senescence previously documented in peripheral tissues.

## Introduction

Senescence, or "to grow old" in Latin, can be observed both systemically and on the level of individual cells. Overall, it can be viewed as a state that is associated with aging, exhibiting a decline in normal function and increased vulnerability to stressors. The concept of cellular senescence (CS) was first introduced more than five decades ago (Hayflick and Moorhead, 1961) based on the finding that cells in culture could only undergo a limited number of divisions (the Hayflick limit). It is generally believed to be an alternate cell fate in the absence of apoptosis (programmed cell death) (Bree et al., 2002). Recently, however, it has become clear that senescence is not solely restricted to the loss of replicative ability, but in fact involves changes in cellular metabolism, epigenetic regulation and gene expression. The prototypical molecular changes that occur during senescence, which include altered morphology, expression of pro-inflammatory cytokines, growth factors and proteases, have collectively been termed the senescence-associated secretory phenotype (SASP) by the Campisi Lab (Coppe et al., 2010a). At present, these phenotypic changes along with increased expression of the cell cycle regulating protein p16(INK4a) and β-galactosidase (β-

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gal) activity are the predominate markers used to identify senescence cells (Carnero, 2013; Salama et al., 2014).

The relationship between CS and organismal aging has only just begun to be explored. Markers of senescence have been found to increase progressively with age in most organisms, including mouse and human tissues (see (van Deursen, 2014) for a review). However, correlation does not necessarily indicate causality. A recent study examined this question directly utilizing transgenic mice in which senescent cells (defined as those expressing p16(INK4a)) undergo apoptosis (Baker et al., 2011). Crossing these mice with a progeroid mouse model (BubR1H/H) reduced age-related phenotypes including sarcopenia, cataracts and loss of adipose tissue (Baker et al., 2011). Also SASP has been suggested to contribute to several age-related diseases including obesity, diabetes, cancer and cardiovascular dysfunction (See (Tchkonia et al., 2013) for a review). These experiments suggest that CS plays a role in age-related conditions in multiple tissues.

The idea that cellular senescence is only an aging-related phenomenon was recently called into question by the discovery of developmental senescence. This research has demonstrated that during embryonic development cells enter a senescent state, as evidenced by β-gal activity, and exhibit SASP (Munoz-Espin et al., 2013; Storer et al., 2013). This distinctly non-aging and non-insult induced occurrence of SASP suggests that SASP and senescence cannot be viewed merely as proliferation arrest and a “side effect” of aging, but is in itself a selective and purposeful mechanism, i.e. a means of clearing unnecessary cells and modulating the tissue microenvironment.

The observation that senescence is not restricted to aging but occurs during normal development and across multiple tissue types raises many questions. Is aging-associated senescence simply a developmental process gone awry? Do all cells senesce through the same mechanisms and subsequently exhibit a similar senescent phenotype? Growth arrest is traditionally viewed as one of the major hallmarks of senescence. How does senescence in this regard apply to post-mitotic cell populations such as fully differentiated neurons, osteocytes, skeletal and cardiac muscle cells? Are there, and if so to what extent, shared phenotypic traits between aging dividing cells (traditionally described as “senescent cells”) and aging post-mitotic cells (traditionally not believed to undergo senescence)?

As interesting studies are emerging on the role of senescence in different age-related pathological conditions, it seems that a particularly understudied field is that of senescence in age-related diseases of the central nervous system (CNS). In addition to age-related cognitive decline, age is the primary risk factor for many neurodegenerative diseases including Alzheimer’s disease (AD), Parkinson’s disease (PD) and frontotemporal dementia (FTD). These diseases are all characterized by dysfunction and death of neurons and glial reactions that create an inflammatory milieu in the affected brain regions. In light of the many recent findings on metabolic and epigenetic alterations in relation to senescence, we will explore different phenotypes and hallmarks of CS, and examine whether similar hallmarks are seen in neurons and glia associated with age-related neurodegenerative disease as well as normal aging.

## Hallmarks of Cellular Senescence

### Pathways to Cellular Senescence

The inducers of a senescent phenotype are typically categorized into three groups (Figure 1): replicative senescence (RS), stress-induced premature senescence (SIPS) and oncogene-induced senescence (OIS) (Kuilman et al., 2010). In brief, RS – the original idea of senescence proposed by Hayflick and Moorhead (Hayflick and Moorhead, 1961) – canonically occurs in response to telomere shortening. This triggers the DNA damage response (DDR) causing activation of the Ataxia-Telangiectasia Mutated (ATM) and Ataxia Telangiectasia and Rad3-related (ATR) pathways that induce cell cycle arrest by activating the cell cycle-regulating protein kinases CDK2 and CDK1, respectively (Di Micco et al., 2008).

In contrast, SIPS occurs independently of telomere length – hence the name premature – and its induction is seen in response to long-term exposure to sub-cytotoxic doses of a number of stressors including oxidative stress (Chen et al., 1995), UVB radiation (Medrano et al., 1995), artificial and inadequate culture conditions ((Ramirez et al., 2001; Sherr and DePinho, 2000) for a review; also see Figure 1). OIS can be considered a special type of SIPS, in which senescence arises due to activation of oncogenes such as *K-ras*, *B-raf*, *PTEN* and *NF1* (Larsson, 2011). Common to all types of senescence is the up-regulation of one or several of the tumor suppressor genes including $p19^{ARF}$, p53, p21, p16(INK4a) and the mitogen-activated protein kinase p38MAPK. In particular, p53 and the p16(INK4a)-Rb signaling pathways play a central role in the achievement of growth arrest regardless of inducer, whereas $p19^{ARF}$ is strongly associated with OIS (Larsson, 2011; van Deursen, 2014). OIS is commonly viewed as a feedback mechanism to suppress oncogenesis, although oncogenic mutations in some cases appear to coopt a senescence-associated inflammatory response (SIR), a component of SASP to further enhance tumor progression (Pribluda et al., 2013).

### Gene Expression and Epigenetic Mechanisms

Altered morphology is one of the major hallmarks of CS across disparate cell types, with cells becoming enlarged and demonstrating an increased cytoplasm to nucleus ratio (Hayflick and Moorhead, 1961; Majore et al., 2009). mRNA and miRNA expression profiles of different human tissues during aging reveal the existence of a common pattern of defined transcriptional and epigenetic changes as well as tissue-specific age-related alterations (ElSharawy et al., 2012; Li et al., 2009; Maes et al., 2009; Ren et al., 2012; Santarosa et al., 2009; Serna et al., 2012).

Gene expression profiles suggest that senescence is a tightly regulated process, with consistent alterations in heterochromatin in CS across tissues. Senescence-associated heterochromatic foci (SAHF) are dense puncta of DNA observed *in vitro* following replicative and stress-induced senescence. They are associated with increased methylation of Lys9 on histone H3 (Bannister et al., 2001). Although SAHF are a frequently observed feature of CS and often used as a marker of CS, a recent report found dissociation between SAHF occurrence and other CS-phenotypes in multiple primary cells and cell lines (Kosar et

al., 2011). Despite this report, it is still unclear how the presence or absence of SAHF and the underlying alterations in methylation modulate features of the global transcriptional profile of CS including growth arrest (reviewed by Adams (Adams, 2007; Zhang et al., 2007)) and SASP. Further research is necessary to discern if global transcriptional phenotypes of CS occur regardless of alterations in heterochromatin or if these phenotypes can be dissociated between different inducers of CS and/or different cell types.

Alterations in H2K4me3 and H3K27me3 methylation are one mechanism through which CS-related changes in the transcriptome can occur (Shah et al., 2013). Altered miRNA expression and disruption of miRNA biogenesis through deletion of the enzyme Dicer, (expression of which declines with age,) can also shift cells into a senescent state (Mori et al., 2012). miRNAs such as miR-29 have been discovered to be master regulators of CS (Hu et al., 2014) and it is possible that individual CS phenotypes such as SASP may also be regulated by specific miRNAs. In *C. elegans* and *Drosophila* perturbed miRNA expression can alter lifespan, modulate lipofuscin accumulation in tissues, accelerate a transcriptional profile associated with aging and reduce age-related neurodegeneration (Boehm and Slack, 2005; Liu et al., 2012). Mounting evidence suggests that miRNAs also play an important role in mammalian CS, aging and neurodegenerative diseases (Abe and Bonini, 2013). However, a single miRNA regulating these processes similarly to those in *C. elegans* and *Drosophila* has not yet been discovered.

SASP is a collective term for the altered expression of secreted cytokines observed in CS. SASP is implicated in inflammation, tissue growth and remodeling. Prominent components of SASP include the cytokines IL-6 and IL-8, MMP-1, MMP-3, fibronectin and laminin B (see (Coppe et al., 2010a; Coppe et al., 2010b) for a review). The general increase in inflammation associated with aging has also been attributed to SASP, although further experiments are necessary to provide conclusive evidence of this relationship. The emerging evidence suggests that there are specific gene expression and epigenetic mechanisms by which CS has a major effect on the cell microenvironment and, through SASP, CS may mediate organism-wide phenotypes such as systemic inflammation. Network analysis of age related genes revealed multiple connections to CS and systemic inflammation, further supporting the role of CS in organismal aging (Tacutu et al., 2010; Tacutu et al., 2011).

## Senescence-Associated Metabolic Alterations

The disruption of energy metabolism and degradation of macromolecules (proteins and lipids) is frequently observed in senescent cells. Although senescent cells are metabolically active and synthesize macromolecules (Blagosklonny, 2011; Blagosklonny and Hall, 2009) there are abnormalities in organelle quality and structure. In particular, the mitochondria of senescent cells are abnormally elongated, likely due to increased expression of mitochondrial fusion proteins such as Fis1, DRP1 and OPA1 (Lee et al., 2007; Mai et al., 2010; Yoon et al., 2006). This altered mitochondrial morphology has been proposed to confer protection against oxidative stress (Mai et al., 2010), but it can also result in proton leakage across the mitochondrial inner membrane leading to enhanced compensatory electron transport (Lee et al., 2007). Increased activity of the mitochondrial pyruvate dehydrogenase, which mediates conversion of pyruvate into acetyl-CoA, has been observed

in OIS constituting another potential mechanism for the higher reactive oxygen species (ROS) levels and AMP/ATP ratio observed in CS (Zwerschke et al., 2003). The onset of CS can be delayed in low-glucose culture conditions, potentially by reducing glucose-mediated protein and DNA damage, although it is not clear whether glycolysis is also altered in senescent cells (Zwerschke et al., 2003). Overall, the reduced energy level and structural as well as functional abnormalities of mitochondria may lead to enhanced generation of DNA damage while inhibiting energy-intensive repair.

The evidence for compromised cellular repair in CS remains controversial. Although DNA damage is an established inducer of CS, the occurrence of impaired DNA repair pathways in CS have not been well studied. Accumulation of unrepairable double strand DNA breaks were reported in senescent cells while their ability to repair radiation-induced damage was unimpaired (Sedelnikova et al., 2004). Thus, CS may be exhibiting a different type of DNA damage rather than impaired DNA repair.

Interestingly, the classical CS marker β-galactosidase (lacZ) staining (at pH 6) likely reflects altered lysosomal mass – the lysosome being the main cell organelle responsible for degradation of damaged macromolecules (Lee et al., 2006). The lipofuscin and α-fucosidase accumulation observed during CS furthermore suggests an increase in lysosomal biomass (Hohn et al., 2012; Singh and Piekorz, 2013). However, it is still controversial if the increase in lysosomal mass indicates enhanced degradation activity or results from impaired lysosomal function. Similarly, reports exist on both enhanced and inhibited autophagy in CS (Grune et al., 2005; Young et al., 2009). Ceramide levels have been shown to increase during CS (Venable et al., 1995), which is interesting since an increase in ceramide concentration can induce a pro-apoptotic autophagy response. Taken together, there appears to be a relationship between autophagy and CS which is understandable in light of the relationship between autophagy and apoptosis.

## Senescence in the CNS

As described above, hallmarks of CS encompass more than just irreversible growth arrest (Figure 2). However, given the typical association of growth arrest with CS, it is interesting to examine whether moderately proliferative cells like astrocytes and oligodendrocyte precursors or even post-mitotic cells like neurons are also subject to CS. Direct experimental evidence for neural cell senescence has been lacking until recently, as some phenotypical markers of senescence have been shown in neurons and astrocytes (Table 1).

A senescence-like phenotype has been observed in Purkinje and cortical neurons in response to DNA damage. The mechanism of induction was similar to classical CS and involved a DDR response, and p21 and p38MAPK activation. Other senescence-associated phenotypes were also observed, including elevated β-gal staining and a SASP-like secretion of pro-inflammatory cytokines (Jurk et al., 2012). A recent study found that cultured cerebellar granule neurons (CGNs) exhibit decreased base excision repair and non-homologous end joining repair over a 5-week time course in culture. This reduction in DDR was correlated to an observed increase in beta-galactosidase activity and intracellular calcium level (Bhanu et al., 2010). These findings suggest that the DNA damage that accumulates in aging neurons

leads to senescence, although this study did not demonstrate a causal relationship between DDR impairment and neuronal senescence. In addition to DDR-induced senescence, aging neurons also exhibit CS phenotypes after exposure to oxidative and metabolic stress (Jurk et al., 2012). Increased beta-galactosidase activity was observed in hippocampal neurons upon prolonged culture of 20–30 days (Dong et al., 2011) and also in the hippocampus of aging rats (Geng et al., 2010). Dong et al. also report on mitochondrial dysfunction in neurons that result in elevated ROS levels (Dong et al., 2011). While these observations suggest mitochondrial impairment plays a role in neuronal senescence, this evidence is correlative and there is an inherent selection bias as typically many neurons die in prolonged culture conditions.

SIRT1, a deacetylase associated with longevity and metabolic regulation, has been shown to attenuate CS in several cell types including neurons. Aging neurons, particularly those that participate in wakefulness activity (hypothalamic orexinergic neurons, locus ceruleus neurons, and mesopontine cholinergic and dopaminergic neurons) have been found to have accumulation of lipofuscin, another commonly used hallmark of CS and indicator of senescence-associated metabolic dysfunction. These aged neurons presented with morphological alteration including reduced neurotransmitter synthesis and dendritic complexity (Panossian et al., 2011). In two neuron-like cell lines (P12 and SH-SY5Y) exposure to the neurotoxin TCDD (2, 3, 7, 8-tetrachlorodibenzo-P-dioxin), which is known to cause mitochondrial dysfunction and accumulation of ROS, also induced a senescence-like phenotype including β-gal staining, increased expression of p16 and p21, reduced p-Rb expression and γ-H2AX foci. TCDD-induced neuronal senescence was dependent on oxidative stress and the senescent phenotype was attenuated by the ROS scavenger N-acetylcysteine (NAC) (Wan et al., 2014). In addition to stress-induced senescence, there is also evidence for CS in neuroblastoma cells as a result of altered gene expression. Knockdown of MECP2, a chromatin-modifying protein that mediates gene silencing increases β-gal staining in a neuroblastoma cell line (Squillaro et al., 2012). In neurons, MECP2 mediates the expression of immediate early gene expression, a set of genes important for synaptic plasticity and memory formation (Deng et al., 2014). This suggests that an increase in β-gal staining could be the result of individual gene expression or a response to an aberrant transcriptional network. While these data raise the possibility of a direct connection between MECP2 status, synaptic plasticity and CS, the experiments were performed in neuroblastoma cells, which are proliferative and thus fundamentally different from neurons.

While the concept of neuronal senescence is new, it is well established that neuronal function declines with advancing age. Disruption of normal calcium homeostasis, with increased intracellular resting levels of $Ca^{2+}$ and an impaired ability to remove excess $Ca^{2+}$ in response to glutamate stimulation is one extensively studied hallmark of aged neurons (Raza et al., 2007; Verkhratsky et al., 1994). The impaired calcium homeostasis may be the result of an age-related reduction in glutathione, a major antioxidant protein that was also found to contribute to the oxidative stress-dependent CS (Belrose et al., 2012). Impaired neuronal calcium homeostasis, neurotransmitter release and cognitive impairment is also likely exacerbated by glial dysfunction, as these support cells of the brain play a central role

in maintaining homeostasis crucial for neuronal function including regulation of metabolites, neurotransmitter uptake and synaptic pruning.

Glial cells include astrocytes, oligodendrocytes and microglia. Microglia are macrophage-like myeloid cells, which serve as the primary innate immune cells of the CNS. As opposed to astrocytes and oligodendrocytes microglia are of non-neural tube origin (Ginhoux et al., 2010). Aging microglia cells show altered morphology distinct from the reactive microglia (Conde and Streit, 2006b). This dystrophic microglia morphology is observed with an increasing frequency in older individuals and in relation to several neuropathologic conditions supporting the notion that dystrophy is age-related and followed by altered functionality of the cells (Conde and Streit, 2006a; Streit, 2006; Streit et al., 2004). Flanary et al. reported that microglia grown *in- vitro* were subject to replicative senescence as assessed by telomere shortening and decreased proliferation (Flanary and Streit, 2004). Additional telomere and telomerase analysis studies *in vivo* support this hypothesis (Flanary et al., 2007; Miller et al., 2007; Miller and Streit, 2007).

Astrocytes and oligodendrocytes originate from CNS stem cells and have an important role in regulating and supporting neuronal function (Rowitch and Kriegstein, 2010). Neural stem cells (NSCs) themselves where shown to undergo senescence in response to extensive proliferation and stressors *in vitro* (Ferron et al., 2004). Interestingly, amyloid-peptide (A) which accumulates in the brain in AD and can be neurotoxic induced NSC senescence possibly through oxidative stress and/or the formylpeptide receptor 2 (FPR2) (He et al., 2013). *In vivo* NSC express several markers of CS with age including telomere shortening, up-regulation of cell cycle genes and ROS accumulation with age (Bose et al., 2010; Ferron et al., 2009).

Differentiated NSC to astrocytes and oligodendrocytes can also undergo CS. Differences exist between these two populations of glial cells with respect to replicative senescence. While astrocytes have been reported to senesce as a result of protracted proliferation (Bitto et al., 2010), oligodendrocyte precursor cells (OPCs) were observed to be resistant to RS *in vitro* (Tang et al., 2001). On the other hand, in OPCs both stress and serum-starvation were shown to induce CS mediated by the esophageal cancer-related gene 4 (Ecrg4). In addition, recombinant Ecrg4 was sufficient to induce CS *in vitro*, and the level of Ecrg4 was reported to increase with age in mice (Kujuro et al., 2010).

More studies have been performed on senescence in relation to astrocytes and suggest that astrocytes are subject to stress-induced CS *in vitro*, with clear similarities to the phenotype observed in senescent fibroblasts: SAHF, p53, p21, p16, SA β-gal activity and secretion of pro-inflammatory cytokines similar to SASP (Bhat et al., 2012; Bitto et al., 2010; Evans et al., 2003) (Figure 2). Due to the prominent role of astrocytes in CNS homeostasis, CS of astrocytes will likely have implications for impaired maintenance of the BBB, regulation of CNS vasculature, neurotransmitter uptake and many other critical CNS functions that are known to decline with age (Sofroniew and Vinters, 2010). Taken together, it appears that both neurons and glia are subject to stress-induced CS as a function of either accumulated DNA damage or oxidative stress, both of which increase during CNS aging and in neurodegenerative disease.

The concept of neuronal CS in response to accumulating stress is emerging (Figure 1). Moreover, it seems that several phenotypes of aging neuronal cells are similar to those observed in proliferative cells (Figure 2). It has also been suggested that neuronal senescence could result from stress due to proteopathies such as aggregates of A or misfolded proteins such as -synuclein (Golde and Miller, 2009). Hence, further research is needed to clarify the matter and determine whether neuronal and glial senescence could be a result of distinct molecular mechanisms and if neuronal CS presents with a unique phenotype that may contribute to age-related decline and neurodegeneration.

## Do Hallmarks of Cellular Senescence Occur in Neurodegenerative Disease?

Senescent cells are characterized by morphological abnormalities, altered gene and protein expression, changes in global methylation patterns and SAHF (Carnero, 2013; Salama et al., 2014; van Deursen, 2014), but do similar abnormalities occur in neurodegenerative disease? Evidence for telomere shortening, an established marker of cellular senescence, in the CNS of aged individuals and AD and PD patients is inconclusive (Eitan et al., 2014), which indicates that RS is unlikely to play an important role in the etiology of these diseases.

Interestingly, similar to senescent cells, increased expression of p16 and p21 were observed in the CNS during aging and in neurons of AD patient tissue (Luth et al., 2000; McShea et al., 1997) (Table 1). However, other cell cycle proteins like cyclin B, cyclin D and PCNA that are not necessarily related to CS are also elevated in neurons of patients with mild cognitive impairment and AD (Yang et al., 2003). It is therefore unknown if these alteration lead to CS or apoptosis and, in that regard, it is interesting that Aβ disrupts signaling by PAK3, a kinase downstream of p21 that mediates neuronal apoptosis and DNA synthesis (McPhie et al., 2003). Inhibition of PAK3 signaling results in disrupted dendritic morphology both in primary hippocampal neurons and *in vivo* in APPswe mice (Zhao et al., 2006). Curiously, in contrast to senescent fibroblasts neurons in AD may exhibit elevated levels of p16 and p21, and an increase in DNA synthesis. This has contributed to the idea that post-mitotic neurons aberrantly enter the cell cycle due to DNA damage, which results in an endpoint of programmed cell death (Kruman, 2004; Kruman et al., 2004). Reduced DNA synthesis in senescent fibroblasts can be restored by blocking both p53 (Gire and Wynford-Thomas, 1998) and p21 (Ma et al., 1999). It is possible that this divergence in DNA synthesis phenotype in AD neurons expressing classical senescence markers could be due to the unique post-mitotic environment of neurons, but more thorough *in vitro* and *in vivo* work will be required to answer this question.

Another feature of both age-related neurological disease and senescence is the expression of p38MAPK and chronic inflammatory signaling. Expression of the p38MAPK in fibroblasts was sufficient to induce SASP by up-regulating NF-κB at the transcriptional level (Freund et al., 2011). This induction of SASP may be independent of DDR and other senescence-inducing mechanisms because inhibition of p38MAPK is sufficient to mitigate the response (Freund et al., 2011). Activity of p38MAPK is increased in neurons of AD patients with neurofibrillary tangles and precedes Aβ plaques in mouse models of AD (Pei et al., 2001; Savage et al., 2002; Sun et al., 2003). Soluble APPα has also been found to activate microglia in a p38-dependent manner (Bodles and Barger, 2005) and histopathological

studies have suggested a role for dystrophic microglia in tau pathology (Streit et al., 2009), further supporting the notion of senescent cells being implicated in AD pathogenesis. Interestingly, p38MAPK hyper-phosphorylation or its inhibition blocks Aβ-induced inhibition of LTP (Li et al., 2003; Wang et al., 2004). It is important to note, however, that p38MAPK also plays a central role in immune activation (Cuenda and Rousseau, 2007) that is unrelated to CS and it is therefore difficult to conclude how much of its observed activity in the CNS is due to CS.

Cytokines such as IL-6 that are characteristic of SASP are found elevated in AD and PD patient tissue and CSF (Bauer et al., 1991b; Blum-Degen et al., 1995; Huell et al., 1995; Wood et al., 1993). Similarly, elevation of IL-6 in the CNS is also observed in normal aging and as a response to chronic psychological stress, as seen for example in AD caregivers (Kiecolt-Glaser et al., 2003). Interestingly, expression of IL-6 in transgenic mice is sufficient to induce neurodegeneration (Campbell et al., 1993). At present it is not clear if the cytokines are produced due to CS or other aspects of disease etiology.

Interestingly, elevated levels of transforming growth factor (TGFβ) mRNA have been observed in AD patient brain tissue (Luterman et al., 2000). TGFβ released by astrocytes increases neuronal expression of the complement protein c1q (Bialas and Stevens, 2013), which can mediate synapse elimination (Stevens et al., 2007) and is known to increase with age in the CNS (Stephan et al., 2013). Hence, AD-related TGFβ signaling could be a central mechanism mediating synapse loss. TGFβ signaling has also been shown to induce senescence *in vitro* (Acosta et al., 2013; Cipriano et al., 2011; Senturk et al., 2010). Furthermore, transgenic mice with elevated astrocyte TGFβ signaling show accelerated Aβ plaque deposition (Mattson et al., 1997; Wyss-Coray et al., 1997), while in an AD mouse model in which TGFβ signaling was blocked, there was a reduction in Aβ pathology (Town et al., 2008). Taken together, these data suggest a direct role for TGFβ and other SASP-related cytokines in both synaptic loss and neurodegeneration.

In AD patient tissue compared to age-matched control subjects a global decrease in euchromatin was observed (Crapper et al., 1979), and another study employed electron microscopy to provide evidence of structural alterations in the nuclear envelope (Metuzals et al., 1988). A more recent study found that tau induces aberrant gene expression through ROS-mediated chromatin relaxation in both human AD patient tissue and in tau transgenic *Drosophila* and mice (Frost et al., 2014). Methylation patterns in AD patient brain tissue have been examined (see (Mattson, 2003) for a review) but have focused mostly on methylation status of specific pathology-related genes rather than global methylation patterns. One study found a global decrease in methylation in entorhinal cortex tissue from AD patients, and a decrease in the methylation maintenance factors MBD2 and DNMT1 (Mastroeni et al., 2010). How this global decrease in methylation relates to the recent observation of senescence-induced methylation “mesas” (H3K4me3 and H3K27me3, e.g. methylation enriched regions) and “canyons” (H3K27me3, e.g. methylation-depleted regions) is unclear, and more rigorous global methylation studies need to be performed in tissue from relevant brain regions of patients with AD or other neurodegenerative disorders. It will also be important to establish changes that occur in brain cells during normal aging.

Lysosomal dysfunction in senescent cells results in two of the most utilized markers of senescence, namely, the accumulation of senescence-associated beta-galactosidase (SA β-gal) and lipofuscin (Carnero, 2013). As noted above, increased β-gal activity was observed in rat hippocampus during aging (Geng et al., 2010). Interestingly, most of the 40 different lysosomal storage diseases frequently result in neurodegeneration (see (Settembre et al., 2008) for a review). Moreover, accumulation of autophagosomes has been observed in several neurodegenerative diseases including AD, PD, Huntington's disease and amyotrophic lateral sclerosis (ALS) (Nixon, 2013; Wong and Cuervo, 2010). In AD the accumulation of autophagosomes appears to result from lysosomal dysfunction and rather than an increase in autophagy initiation (Lee et al., 2010). Ceramide has also been shown to increase in senescent cells as well as aging brain and brains of AD and ALS patients (Cutler and Mattson, 2001; Cutler et al., 2002; Haughey et al., 2010). More work is needed to elucidate the connection between ceramide, autophagy, apoptosis and CS in brain cells.

Similar to the alterations observed in senescence, cellular energy metabolism is perturbed in AD and PD which manifests as altered mitochondrial function and increased ROS production ((Demetrius and Driver, 2013) see (Mattson et al., 1999) for a review). Evidence from *in vitro* cybrid experiments in which native mitochondria are replaced with AD and PD patient mitochondria demonstrated enlarged mitochondria, altered calcium homeostasis and reduced mitochondrial membrane potential as measured by JC-1 when compared to cells populated with mitochondria from age-matched controls (Sheehan et al., 1997a; Sheehan et al., 1997b; Trimmer et al., 2000). A more recent study examining levels of mitochondrial fission and fusion proteins found a reduction of mitochondrial fission proteins (Drp1, Opa1, Mfn1, Mfn2) and an increase in the fusion protein Fis1 (Wang et al., 2009). In PD diminished mitochondrial function and increased ROS clearly plays a role in pathology as familial PD mutations such as DJ-1, PINK1 and Parkin1 directly impact mitochondrial health and function, and environmentally-induced PD is typically due to toxins that act on mitochondria such as MPTP (see (Scarffe et al., 2014) for a review). It is possible that the altered mitochondrial function and morphology found in AD tissue and models could be a consequence of Aβ proteopathy (Mattson and Goodman, 1995; Wang et al., 2009) or mutations in genes such as presenilin-1 (AD, (Keller et al., 1998)) or DJ-1 (PD, (Wang et al., 2012)) rather than a consequence of neuronal or glial senescence. While the etiology still needs further investigation, it is clear that brain cells in both AD and PD share similar mitochondrial phenotypes to peripheral senescent cells including altered mitochondrial morphology and enhanced ROS production.

## Conclusions

Hitherto, limited evidence exists on whether post-mitotic neurons can enter into a senescent state. The potential role of neuronal and glial senescence in neurodegenerative disease is similarly underexplored. However, evidence is beginning to emerge. In both AD and PD there is evidence for several CS hallmarks including: aberrant expression of cell cycle proteins (Luth et al., 2000), nuclear abnormalities (Metuzals et al., 1988), lysosomal and autophagic dysfunction (Nixon, 2013), impaired mitochondrial function that leads to enhanced ROS generation (Mattson et al., 1999), and production of pro-inflammatory cytokines such as IL-6 (Wood et al., 1993). While it is still possible that this collection of

senescent-like phenotypes found in CNS cell populations of aging, AD and PD brains could all be due to unique aspects of disease etiology, it is suggestive that CS does indeed take place in the CNS during normal aging as well as in age-related disease. Currently, it is not clear whether the observed senescent-like phenotypes occur in NSCs, glial cells, neurons, or all of these cell types. It seems likely that CS in the CNS is predominantly of the SISP type as even glia cells are not highly proliferative compared to peripheral cells that undergo RS. The potential stress origin of CNS CS may also explain the increase in hallmarks observed during neurodegenerative disease. The recent discovery, that senescence is not solely an age-related process but in addition a developmental mechanism for clearance of unnecessary cells and recruiting an immune response re-frames the process of CS (Munoz-Espin et al., 2013; Storer et al., 2013). In this regard it may be viewed as a signal for recruiting blood-borne immune cells ((Schwartz and Shechter, 2010) for a review), which can exert either beneficial or devastating effects during neurodegenerative diseases. In addition, CNS senescence could be a downstream feature activated as a result of proteopathy-inducing genetic factors or environmental insults that occur with aging and not necessarily a causal force in neurodegeneration. Finally, the possibility that CS in brain aging and neurodegenerative disorders represents an adaptive response should be investigated.

In summary, CS is characterized by more than just cell cycle arrest serving as a tumor suppressor mechanism. Emerging evidence indicates that CS take place in the aging brain, and probably even in post-mitotic neurons. Current evidence indicates that at least several of the neuronal senescent phenotypes are similar to those observed in proliferative cells, while others, like calcium homeostasis may be neuron-specific. The contribution of CS to brain aging and age-related neurodegenerative disease is still not clear. Further investigation of senescence markers in the aging brain and in tissue samples from patients suffering from neurodegenerative diseases can elucidate the type of CS cells of the CNS undergo and the underlying mechanisms, and may help assess the contribution of senescence to disease etiology.

## Future Directions

Our review of the literature has revealed that only indirect evidence exists to suggest the possibility of CS in post-mitotic cells such as neurons. We feel that experiments addressing four critical questions will shed light on the contribution of CS to CNS aging and neurodegenerative disease:

Does inhibiting cellular senescence in the brain in either neuronal or glial populations attenuate age-related cognitive decline and progression of neurodegeneration?

Conversely, does artificially inducing senescence in either neuronal or glial populations produce enhanced cognitive decline and accelerated neurodegeneration?

Are the common phenotypes observed in both classical CS and neurodegenerative disease representative of a common mechanism of senescence or simply indicators of cellular stress and dysfunction?

What is the occurrence of neuronal senescence *in vivo* under normal cognitive aging and in neurological disease?

Recent developments have now enabled researchers to answer these critical questions. Progress in single cell analysis techniques now enables researchers to examine neuronal populations expressing SA -gal using techniques like single-cell PCR to determine similarities between neuronal senescence and senescence in mitotic populations. A more thorough understanding of the mechanisms involved in CNS senescence will be critical to understanding the contribution of CS to age-related neurodegenerative disease. Such work will contribute to understanding of age-related cognitive decline and could help in the development of novel therapeutic interventions in age-related neurodegenerative disease.

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## Abbreviations

| | |
|---|---|
| **A** | Amyloid-peptide |
| **AD** | Alzheimer’s disease |
| **ALS** | Amyotrophic lateral sclerosis |
| **ATM** | ataxia telangiectasia mutated |
| **ATR** | ataxia telangiectasia and Rad3-related |
| **β-gal** | β-galactosidase |
| **CGNs** | cerebellar granule neurons |
| **CNS** | central nervous system |
| **CS** | cellular senescence |
| **CSF** | cerebrospinal fluid |
| **DDR** | DNA damage response |
| **Ecrg4** | esophageal cancer-related gene 4 |
| **FTD** | frontotemporal dementia |
| **HD** | Huntington’s disease |
| **LTP** | long-term potentiation |
| **miRNA** | microRNA |
| **NSCs** | neural stem cells |
| **OIS** | oncogene-induced senescence |
| **OPCs** | oligodendrocyte precursor cells |
| **PD** | Parkinson’s disease |
| **ROS** | reactive oxygen species |
| **RS** | replicative senescence |

| | |
|---|---|
| **SA β-gal** | senescence-associated β-gal |
| **SAHF** | senescence-associated heterochromatic foci |
| **SASP** | senescence-associated secretory phenotype |
| **SIPS** | stress induced replicative senescence |
| **SIR** | senescence-associated inflammatory response |
| **TGFβ** | transforming growth factor |

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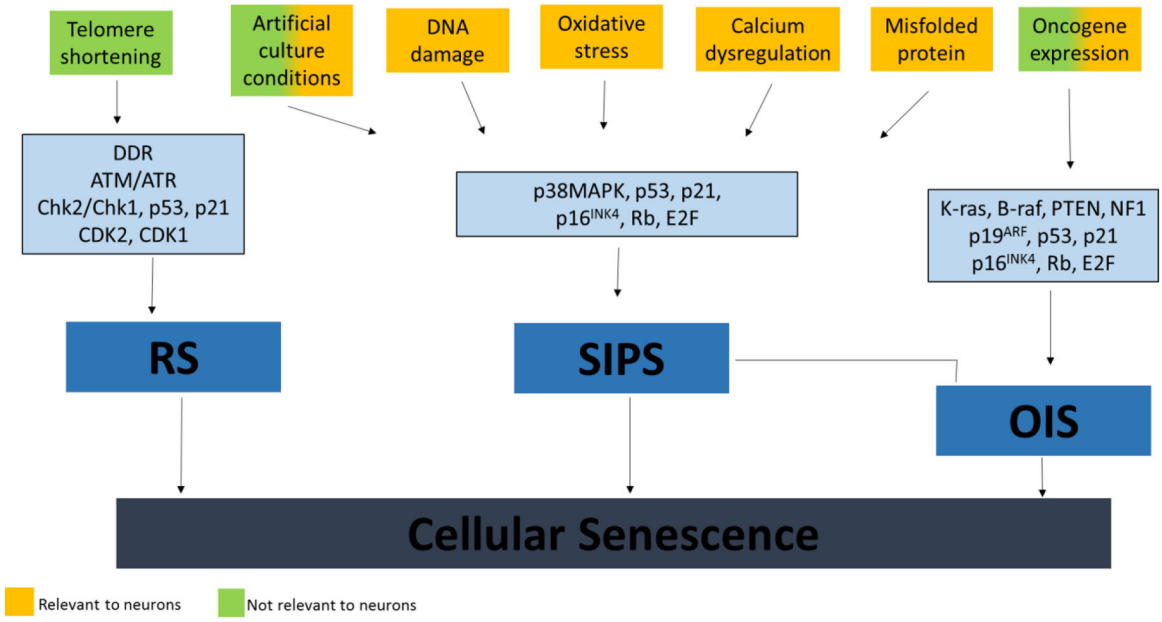


**Figure 1.**

Pathways to cellular senescence. This figure summarizes the different stressors (top layer) and molecular mechanisms (top middle layer) involved in mediating each type of senescence (bottom middle layer) collectively termed “cellular senescence” (bottom layer). RS – replicative senescence; SIPS – stress induced premature senescence; OIS – Oncogene-induced senescence. Yellow color indicates a type of stressor that is relevant to neurons. Green color indicates a type of stressor only relevant to other cell types.

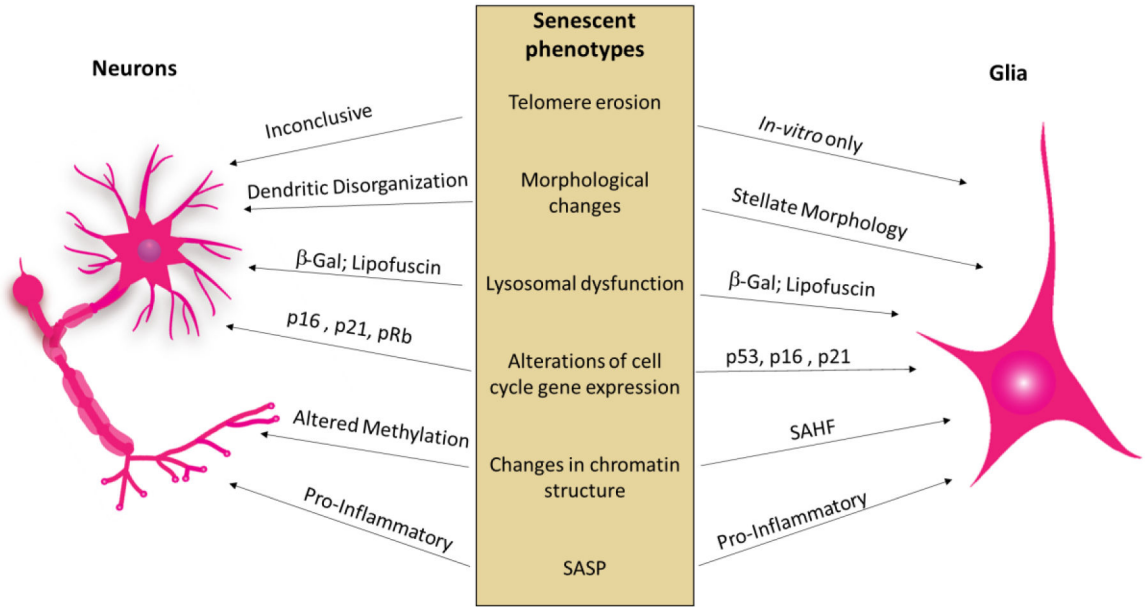


**Figure 2.**

Evidence for senescence in CNS cell populations. Both aging neurons (left) and glial cells (right) show phenotypic traits characteristic of cellular senescence (middle).

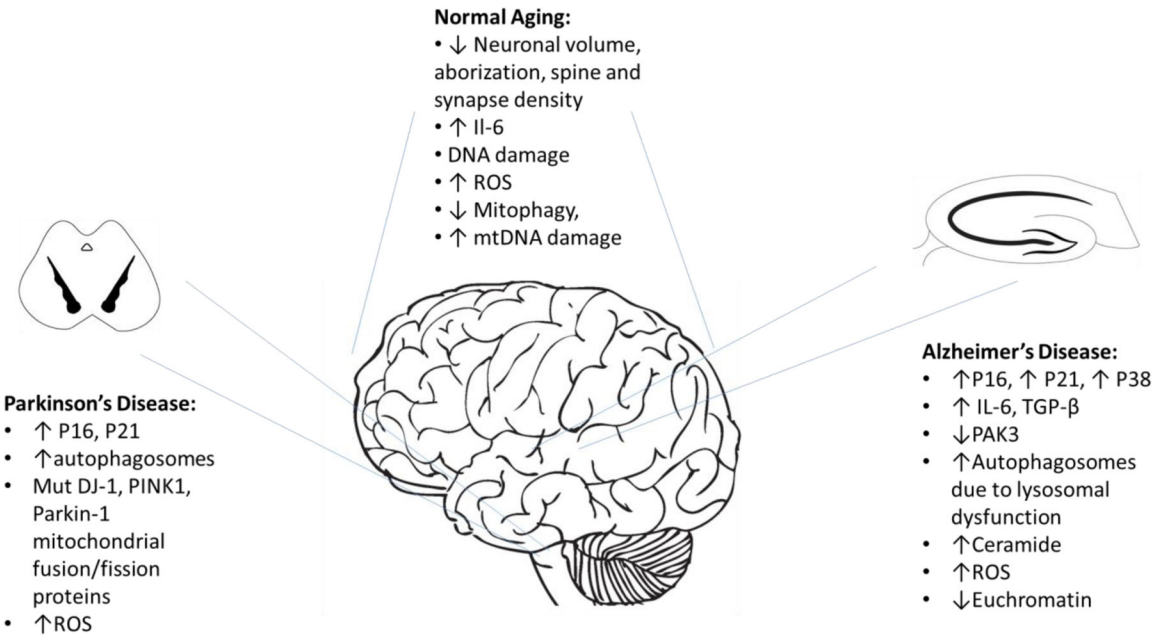


**Figure 3.**

Evidence for senescence in neurodegenerative disease. Several hallmarks of cellular senescence are found to be increased in normal aging as well as in neurodegenerative disorders such as Alzheimer's and Parkinson's diseases.

**Table 1**

Occurrence of various phenotypes associated with cellular senescence in fibroblasts, astrocytes and neurons in normal aging, and in Alzheimer's and Parkinson's diseases.

| Phenotype | Fibroblasts | Astrocytes | Neurons | Normal Aging | Alzheimer's Disease | Parkinson's Disease |
|---|---|---|---|---|---|---|
| Telomere shortening | + (Bodnar et al., 1998; Harley et al., 1990) | − (Evans et al., 2003) | | − (Cherif et al., 2003) | **Inconclusive** (Eitan et al., 2014) | **Inconclusive** (Eitan et al., 2014) |
| β-galactosidase | + (Dimri et al., 1995) | + (Bhat et al., 2012; Bitto et al., 2010; Evans et al., 2003) | + (Jurk et al., 2012) (Squillaro et al., 2012) (neuroblastoma) (Wan et al., 2014) (PC12) | + (Geng et al., 2010) | + (Tiribuzi et al., 2011) | + (van Dijk et al., 2013) (CSF) |
| Lipofuscin | + (Sitte et al., 2001; Vogt et al., 1998) | + (Kim et al., 2002) | + (Panossian et al., 2011) | + (Kim et al., 2002; Oenzil et al., 1994) | + (Hirai et al., 2001) | + (Braak et al., 2003) |
| Morphological changes | **↑ cytoplasm/nucleus, flat** (Wang and Gundersen, 1984) | **Enlarged cell body, flat** (Yamada et al., 2008) | **↑ cytoplasm/nucleus** (Ledda et al., 2000; Peinado et al., 1993) **Dendritic atrophy and regression** (Rafols et al., 1989) **↓ myelination** (Peters et al., 2000) | | **Dendritic spine defects** (Zhao et al., 2006) | |
| Nuclear disorganization | **↓ Lamin B** (Freund et al., 2012; Shimi et al., 2011) | **↑ Size, nuclear bodies, nucleolar disorganization** (Berciano et al., 1995) | **No change** (Lesuisse and Martin, 2002) **↑ nuclear area/IOD** (Rodrigues et al., 2014) | | | |
| Cell cycle regulating genes | **p53** (Kulju and Lehman, 1995) **p16(INK4a)** (Alcorta et al., 1996) **$p21^{WAF1}$** (Alcorta et al., 1996; Noda et al., 1994) **E2F-1 loss** (Dimri et al., 1994) **Activated Rb** (Narita et al., 2003) | **p53, $p21^{WAF1}$** (Bitto et al., 2010; Evans et al., 2003) **p16(INK4a)** (Bitto et al., 2010) (Bhat et al., 2012) (both *in vivo* and *in vitro*) | **p16(INK4a), p21, pRb** (Wan et al., 2014) | **p16(INK4a)** (Bhat et al., 2012) | **p16(INK4a)** (Bhat et al., 2012; Luth et al., 2000; McShea et al., 1997) **$p21^{RAS}$** (Luth et al., 2000) **↑ CDK4** (McShea et al., 1997) **Hyper-P Rb (inactive)** (Ranganathan et al., 2001) **PAK3 inh.** (McPhie et al., 2003) | **Hyper-P Rb (inacitve)** (Jordan-Sciutto et al., 2003) |
| p38MAPK | + (Freund et al., 2011; Iwasa et al., 2003) | + (Bhat et al., 2012) | + (Jurk et al., 2012) | − (Sun et al., 2003) | + (Pei et al., 2001; Savage et al., 2002; Sun et al., 2003) | |
| SASP | **IL-6, IL-8, MMP-3** (Rodier et al., 2009) **NF B** (Freund et al., 2011) | **IL-6, MMP-1** (Bhat et al., 2012) **IL-1β** (Yu et al., 2002) | **IL-6** (Jurk et al., 2012) | **MMP-1** (Bhat et al., 2012) | **IL-6** (Bauer et al., 1991a; Blum-Degen et al., 1995; Huell et al., 1995; Luterman et al., 2000; Wood et al., 1993) **IL-1β (CSF)** (Blum-Degen et al., 1995) | **IL-6, IL-1β** (Blum-Degen et al., 1995) (CSF) |

| Phenotype | Fibroblasts | Astrocytes | Neurons | Normal Aging | Alzheimer's Disease | Parkinson's Disease |
|---|---|---|---|---|---|---|
| | | | | | **TGF-β** (Luterman et al., 2000 Luterman et al., 2000) **MMP-1** (Bhat et al., 2012) | |
| SAHF | + (Bitto et al., 2010; Narita et al., 2003) | + (Bitto et al., 2010) | | | | |
| Retrotransposons | **↑ Alu, SVA, L1** (De Cecco et al., 2013) | | | **↑ R1, R2, gypsy** (Li et al., 2013) | | |
| DNA methylation | **Global hypomethylation, ↓ DNMT1, ↑MBD2** (Zhang et al., 2008) | | | | **↓ DNMT1, MBD2** (Mastroeni et al., 2010) | |
| Histone modifications | **SIRT6, H3K9** (Michishita et al., 2008) **H3K9me3, H3K27me3** (Chandra et al., 2012) | | **γH2AX** (Jurk et al., 2012) (Wan et al., 2014) (PC12) **↓ SIRT1** (Panossian et al., 2011) **↓ heterochromatic areas, ↓ H3K9me3, ↑ H3K9Ac** (Rodrigues et al., 2014) | | **↓ euchromatin fraction** (Crapper et al., 1979) | |
| Autophagy-lysosomal dysfunction | **↓ Atg7, Atg12, Lamp2** (Kang et al., 2011) | | | **↓ autophagy** (Lipinski et al., 2010) | **↑ autophagy, ↓ lysosomal degradation** (Lipinski et al., 2010) **↓ membrane impermeability** (Yang et al., 1998) **↑ autopha-gosomes** (Lee et al., 2010) | **↓ LAMP1, Cathepsin D, HSP73** (Chu et al., 2009) **↓ Cathepsin E ↑ α-fucosidase** (van Dijk et al., 2013) (CSF) |
| Mitochondrial dysfunction | **↑ MtDNA damage** (Chen et al., 1995) | **↑ ROS production** (Pertusa et al., 2007) **↓MMP** (Lin et al., 2007) | **↑ ROS production** (Jurk et al., 2012) (Wan et al., 2014) (PC12, SH-SY5Y)) **↑ mitochondrial size** (Martinelli et al., 2006) | **↑ MtDNA** (Mecocci et al., 1993) **↓ α-subunit F1 ATP synthase** (Lu et al., 2004) | **↑ MtDNA damage, Enlarged morphology, ↓MMP** (Trimmer et al., 2000) **↑ ROS production, ↓ Complex IV activity** (Sheehan et al., 1997a) **↓ Drp1, OPA1, Mfn1, Mfn2 ↑ Fis1** (Wang et al., 2009) | **↑ MtDNA Damage, Enlarged morphology, ↓MMP** (Trimmer et al., 2000) **↓ Complex I activity** (Sheehan et al., 1997b) **Mut DJ-1, PINK1, Parkin1** (Bonifati, 2014) |