Review

Ageing, Cellular Senescence and Neurodegenerative Disease

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Abstract: Ageing is a major risk factor for developing many neurodegenerative diseases. Cellular senescence is a homeostatic biological process that has a key role in driving ageing. There is evidence that senescent cells accumulate in the nervous system with ageing and neurodegenerative disease and may predispose a person to the appearance of a neurodegenerative condition or may aggravate its course. Research into senescence has long been hindered by its variable and cell-type specific features and the lack of a universal marker to unequivocally detect senescent cells. Recent advances in senescence markers and genetically modified animal models have boosted our knowledge on the role of cellular senescence in ageing and age-related disease. The aim now is to fully elucidate its role in neurodegeneration in order to efficiently and safely exploit cellular senescence as a therapeutic target. Here, we review evidence of cellular senescence in neurons and glial cells and we discuss its putative role in Alzheimer’s disease, Parkinson’s disease and multiple sclerosis and we provide, for the first time, evidence of senescence in neurons and glia in multiple sclerosis, using the novel GL13 lipofuscin stain as a marker of cellular senescence.

Keywords: neurodegeneration; cellular senescence; ageing; Alzheimer’s disease; multiple sclerosis; Parkinson’s disease; lipofuscin; SenTraGor™ (GL13); senolytics

1. Ageing and Neurodegeneration

Ageing is a universal process characterized by the accumulation of biological changes that lead to the organism’s functional decline over time. Human ageing is accompanied by a gradual build-up of cognitive and physical impairment and an increased risk of developing numerous diseases including cancer, diabetes, cardiovascular, musculoskeletal and neurodegenerative conditions. Age-related disability and morbidity adversely affect the quality of life; they are ultimately associated with an increased risk of death and bear dire consequences for the individual, the family and society.

Ageing is the most important risk factor for the development of neurodegenerative disease and typically, most neurodegenerative disorders manifest in the elderly [1]. The annual incidence of Alzheimer’s disease (AD) has been shown to increase exponentially with advancing age [2,3]. Notably, Down syndrome, a progeroid condition, has been associated with AD, and mouse models of premature ageing have been reported to overproduce Aβ and show impaired learning and memory [4–7]. Incidence of Parkinson’s disease (PD), the second most common age-related neurodegenerative condition also increases with age [8,9]. The great majority of AD and PD cases are sporadic and typically manifest at a much older age than hereditary ones. Despite the differences in pathology...
among the two conditions, they are both typical neurodegenerative diseases characterized by chronic progressive loss of neurons and their synaptic connections manifesting with gradual functional decline [4]. But age is a recognized risk factor even for inflammatory demyelinating conditions such as multiple sclerosis (MS), which also has a strong neurodegenerative component [10]. Age is the strongest predictor for the transition from the relapsing phase of MS, which is primarily inflammatory to the secondary progressive phase of the disease, which is thought to be mainly neurodegenerative [10,11].

Although research on the biology of mammalian ageing has recently attracted much attention, our understanding of its underlying mechanisms remains poor. It has been hypothesized that failure of repair mechanisms leads to accumulation of cellular and molecular damage that drives ageing [12]. Accumulating damage is thought to occur inherently in a random manner, which explains the great diversity in ageing phenotypes, even in monozygotic twins [13]. The interplay among the genetic background, environmental factors and the stochastic nature of age-related accumulation of irreparable damage to the DNA of the organism may also determine the likelihood of developing a particular age-related disease. Genomic instability, telomere attrition, loss of proteostasis, dysregulated nutrient sensing, mitochondrial dysfunction, stem cell exhaustion, altered cellular communication and excessive cellular senescence have all been recognized as hallmarks of ageing [14]. Cellular senescence is a process triggered by irreparable DNA damage that underlies normal ageing. Senescent cells become more abundant with ageing and a growing body of evidence suggests that their accumulation may contribute to pathogenesis of age-related diseases. Here, we review the data that support a role for cellular senescence in neurodegeneration, with special focus on AD, PD and MS.

2. Cellular Senescence

Cellular senescence is a homeostatic response aiming to prevent the propagation of damaged cells and neoplastic transformation [15]. Apart from its beneficial role as an anti-tumour response, physiological roles for cellular senescence have also been identified during development [15,16], in adult megakaryocytes, syncytiotrophoblasts, wound healing and placental natural killer lymphocytes [17–19]. However, several lines of evidence indicate that cellular senescence also contributes to the loss of function associated with ageing and age-related disease [20]. According to the original observations by Hayflick and Moorhead (1961), when cultures of normal human fibroblasts were passaged serially they underwent stable cell cycle arrest that was accompanied by stereotypical phenotypic changes [21]. This form of cellular senescence, termed replicative senescence constitutes a particular type of cellular senescence and is associated with telomere shortening with successive cell cycles. Nevertheless, besides telomere shortening, there are many more triggers of cellular senescence including aberrant oncogene activation (oncogene-induced senescence-OIS) [22], stress-induced (stress-induced premature senescence-SIPS) due to oxidative stress [23], ionizing radiation [24], DNA-damaging chemotherapy [25], hyperoxia [26], impaired autophagy [27] or other stressors and mitochondrial dysfunction [28]. Most of these triggers lead to telomeric or non-telomeric DNA damage or altered chromatin structure and typically activate the DNA damage response (DDR) [29,30], although cellular senescence in vitro may also occur without detectable DDR [31,32]. When the cell’s repair mechanisms become overwhelmed, the activated DDR elicits cellular senescence via phosphorylation of p53 [33].

Unlike apoptosis, senescent cells remain viable and metabolically active [30]. Although senescent cells can be recognized by T helper cells and cleared by macrophages and natural killer lymphocytes [27,34,35], their number has been shown to increase with normal ageing in tissues of humans, primates and rodents [36,37]. Models of accelerated cellular senescence show premature ageing and increased incidence of age-related pathologies, suggesting that accumulating senescent cells contributes to ageing-related functional compromise and predisposes to age-related disease [38]. The features of senescent cells that constitute the senescent phenotype may be responsible for their putative detrimental effects in ageing and ageing-associated neurodegenerative disease.
3. The Senescence Phenotype

Senescent cells exhibit a multitude of cellular and molecular changes that are neither specific nor pathognomonic of the senescent state. Evidence suggests that the cellular and molecular features of senescence depend on both the triggering stimulus and the affected cell type [23]. Although the senescence phenotype of nervous system cells has not been extensively studied, the key features of senescence are described below:

Typically, senescent cells exhibit permanent cell cycle arrest, which is thought to be regulated by p16<sup>INK4A</sup> and p53-p21-RB (retinoblastoma). Increased expression of p53 upregulates expression of CDKí(cyclin-dependent kinase inhibitor) p21, which initially arrests the cell cycle. p16<sup>INK4A</sup> mediates permanent cells cycle arrest by inhibiting CDK4 and CDK6, which leads to RB hypophosphorylation and blocks entry to S phase [39,40].

Another key feature of cellular senescence is the senescence-associated secretory phenotype (SASP), which is dependent on p38MAPK (p38 mitogen-activated protein kinases), NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), cGAS(cyclic GMP-AMP synthase)/STING(stimulator of IFN genes), NOTCH and mTOR(mammalian target of rapamycin) signalling [38–42]. SASP consists of chemokines, cytokines, growth factors and metaloproteinases [43]. These factors are primarily proinflammatory and act in a paracrine and autocrine manner [44,45], although immunosuppressive mediators have also been identified as part of SASP [46,47]. Recent data indicate that SASP may also involve small extracellular vesicles in a p53-dependent manner [48–50].

Cellular senescence is also characterized by resistance to apoptotic death, which appears to be largely controlled by the p53 stress response pathway. Both p53 levels and p53 post-translational modifications [51] seem to have a role in determining the senescent cell fate while conferring resistance to apoptosis. Accumulation of intermediate levels of p53 has been reported to favor the expression of anti-apoptotic bcl-2 family proteins [52]. Nonetheless, p21 has also been shown to be capable to directly inhibit caspase 3 and apoptosis [53].

Cellular senescence is associated with changes in cellular metabolism. These include upregulation of lysosomal senescence-associated β-galactosidase, a shift from oxidative phosphorylation to glycolysis [54,55] and accumulation of lipofuscin in the cytoplasm [56,57]. Lipofuscin accumulation has been reported as a key feature of cellular senescence that can be used to positively identify senescent cells [57,58]. A recent metabolomic analysis of cultured doxorubicin-treated breast cancer cells revealed that tricarboxylic acid cycle, pentose phosphate pathway, and nucleotide synthesis pathways were significantly upregulated, whereas fatty acid synthesis was reduced [59].

Altered mitochondrial function appears essential in mediating the senescence phenotype. RNA sequencing analysis has shown that a great number of senescence-associated changes involve the mitochondria and Akt (protein kinase B), ATM (ataxia-telangiectasia mutated) and mTORC1 phosphorylation have been shown to link DDR with mitochondrial biogenesis [60]. Morphological changes in mitochondria are also seen in senescent cells [20,61]. In addition, impaired mitophagy seems to explain accumulation of dysfunctional mitochondria (senescence-associated mitochondrial dysfunction—SAMD) seen in cellular senescence [62,63]. Although mitochondria are not the sole source [63], they are a major generator of ROS, important for both cellular signaling and SASP [47,64–66].

Several epigenetic modifications are also common in cellular senescence. Defects in pericentric heterochromatic silencing at mammalian centromeres, normally regulated by SIRT6 (sirtuin 6) have been described [67]. SIRT6 belongs to the sirtuin protein family, whose function has been linked to longevity [68]. Micro-RNAs, a subclass of regulatory, non-coding RNAs that participate in regulation of cellular senescence may also be epigenetically modified [69]. Chromatic alterations such as senescence-associated heterochromatin formation (SAHF) may accompany cellular senescence in some settings with deactivation of proliferation-related genes [70–73]. Cellular senescence may also lead to changes in the organization of nuclear lamina and down regulation of lamin B1 with implications for nuclear morphology and gene expression [74].
Finally, senescent cells also undergo morphological changes. Cells become larger and flattened out and acquire an irregular shape. These alterations are more prominent in vitro than in vivo and appear to be caused by cytoskeletal rearrangements [75,76] and changes in cell membrane composition [77]. Senescent cells exhibit increased unfolded protein response (UPR), indicative of endoplasmic reticulum (ER) stress [76,77]. The ATF6α pathway of UPR appears to be responsible for both increasing ER size associated with ER stress and regulating the shape and size of senescent cells [76,77].

4. Markers of Cellular Senescence

To better understand the role of senescent cells in physiological and pathological conditions, it is essential to be able to detect them in vitro and in vivo [15]. So far, research on cellular senescence has been hindered by our lack a universal, specific and widely applicable marker of cellular senescence. Here, we discuss the most commonly used markers of senescence:

- **p16$$^{INK4a}$$**: This member of the INK4a family is a cyclin D-dependent kinase CDK4 and CDK6 inhibitor, which prevents the phosphorylation of the retinoblastoma protein (Rb), therefore leading to suspension of the cell cycle before the S-phase [78,79]. Increased levels of p16$$^{INK4a}$$ have been documented in aged and stressed tissues, compared to younger, healthy tissues, whereas the removal of p16$$^{INK4a}$$-expressing senescent cells in mice prevented or delayed tissue dysfunction and age-related disorders [38,80]. This evidence has established p16$$^{INK4a}$$ as a widely-accepted marker of ageing and cellular senescence [81,82]. Limitations include poor detection of p16$$^{INK4a}$$ in mice by the currently available antibodies using immunohistochemistry [79], as well as some situations, where p16$$^{INK4a}$$ levels are increased, in the absence of other signs of cellular senescence [81,83,84].

- **p21$$^{CIP1/WAF1/SDI1}$$**: p21 is a member of the second group of CDK inhibitors, namely the CIP/KIP (CDK interacting protein/kinase inhibitory protein) family and can inhibit a variety of CDKs [85]. Apart from its role in cellular senescence, it is a key mediator in several biological functions, including cell cycle arrest, cell death, DNA repair processes and even reprogramming of differentiated somatic cells into pluripotent stem cells [86]. In the context of senescence, stress-induced p53 activates p21 in order to trigger cell cycle arrest [39]. Although both p21 and p16$$^{INK4a}$$ upregulation lead to cell cycle arrest, they act through different pathways and have distinct roles in the induction and progression of cellular senescence [36].

- **SA-β-gal**: The activity of β-galactosidase detectable at pH 6.0, which is measured using in situ staining with the chromogenic substrate X-gal [senescence-associated β-galactosidase activity (SA-β-gal)] is today the most widely used biomarker for detecting senescent cells [79,87]. The lysosomal enzyme β-galactosidase encoded by the GLB1 (galactosidase beta 1) gene, is the source of SA-β-gal activity and it can, therefore, be elevated in any situation with increased lysosome number or activity [54,88]. It has also been reported that certain cell culture conditions can increase the level of SA-β-gal, giving a false positive result [88,89]. Another drawback of the SA-β-gal assay is that it can only be used on fresh or frozen tissues and not on formalin-fixed paraffin-embedded archival tissue samples, which significantly limits its spectrum of application [15,57].

- **Lipofuscin**: Intracellular lipofuscin aggregates consist of oxidized protein and lipid degradation residues and metal cations that cannot be degraded by lysosomal enzymes. Lipofuscin accumulates with age and its accumulation is a documented hallmark of senescent cells [90–92]. GL-13 (SenTraGor®) is a biotinylated chemical compound derived from Sudan Black-B that specifically and strongly binds to lipofuscin [57,58]. Its ability to detect lipofuscin, not only on fresh tissues, but also on formalin-fixed paraffin-embedded archival samples and biological fluids gives a new perspective in the field of senescence markers [57,58,90,93].

The fact that senescence can be induced by different stimuli and is mediated by several diverse mechanisms, as well as the drawbacks of each senescence marker, has led most researchers to abandon
the single marker approach and rather utilize a combination of different biomarkers. However, to date, there is no consensus on the optimal combination of markers to detect senescence in vivo and in vitro [23,28,79].

5. Cellular Senescence and Its Putative Role in Neurodegeneration

A primary causative role of cellular senescence in neurodegenerative disease is highly unlikely given the great diversity which characterizes ageing-related neurodegenerative pathologies. However, cellular senescence may still substantially contribute to the pathogenesis of neurodegenerative disease and thereby determine disease susceptibility, age at disease presentation and rate of progression. Three mechanisms could explain the putative role of cellular senescence in neurodegeneration:

- **Promotion of chronic inflammation:** Senescence-associated secretory phenotype (SASP) converts senescent cells into continuous sources of pro-inflammatory mediators, reactive oxygen species and metalloproteinases [43]. Senescent cells may sustain a proinflammatory milieu, which can be damaging for neighboring cells or “contaminating” in the sense of converting neighboring cells to senescent ones in a paracrine manner [44,94,95]. Interleukin-6, a typical SASP mediator, is upregulated in the aged brain and in AD [96–98] and its overexpression has been shown to drive neurodegeneration in vivo [99]. The ageing brain has higher background levels of low-grade inflammation primarily in the form of dystrophic microglia and increased levels pro-inflammatory cytokines and other mediators, a state known as inflammaging [100–102]. SASP-related mediators from increased numbers of senescent cells may be what underlies inflammaging [103,104]. There are many links between inflammaging and AD and PD pathologies [105,106], which suggest that SASP may contribute to the pathogenesis of neurodegeneration and may determine disease susceptibility or aggravate the course of the disease.

- **Exhaustion of the regenerative capacities of the nervous system:** There is evidence of neurogenesis from adult neural stem cells deriving from the subventricular zone (SVZ) and the subgranular zone (SGZ) of the hippocampal dentate gyrus, that can give rise to neurons, oligodendrocytes and astrocytes [107,108]. Ageing has been shown to significantly reduce adult hippocampal neurogenesis [109]. Cell cycle arrest of adult progenitor cells in the context of cellular senescence may reduce the regenerative capacities of the CNS. This notion is supported by recent in vivo evidence from the BUBR1 progeroid mouse model in which adult progenitor proliferation was impaired in the SGZ and SVZ in an age-dependent manner [110]. Although the role of adult neurogenesis in AD remains contentious, studies from animal models indicate that ablation of adult neurogenesis exacerbates memory deficits and upregulates hyperphosphorylated tau [111], whereas implantation of human neural stem cells alleviates memory deficits and AD pathology [112]. Furthermore, oligodendrocyte progenitor cells (OPCs) are a population of adult stem cells responsible for mediating CNS myelin repair in demyelinating conditions such as MS [113,114]. However, despite remyelination being very efficient at the early stages of the disease, this process gradually fails over time [115,116]. Evidence from MS and its animal models suggests that remyelination can protect demyelinated axons and even correlates with greater age at death, whereas chronically demyelinated axons are prone to degeneration [117,118]. Inability to replenish adult progenitor cells due to cellular senescence could render the CNS susceptible to neurodegeneration.

- **Loss of function:** The functional state of senescent cells has not been fully elucidated. However, cell-cycle arrest, changes in gene expression and phenotypic changes that accompany cellular senescence constitute serious restrictions in the functionality of different cell types [119]. The number of senescent cells increases with age [30]. At the same time it must be noted that ageing is associated with loss of brain cells to an extent which may amount to up to 0.4% of brain volume, annually [120]. The processes that lead to loss of brain cells with normal ageing are unclear. Both apoptotic and senescent cells can be cleared by the immune system in a highly regulated manner [35]. Thus, brain volume loss may at least partly be due to immune-mediated
clearance of senescent cells. It is also conceivable, that when the number of dysfunctional senescent cells exceeds a certain threshold in a brain with reduced reserves due to age-related cell loss, nervous tissue function is likely to become compromised. Senescent cell accumulation may occur preferentially in some brain regions e.g., substantia nigra, that are more susceptible to particular stressors, which could explain the ensuing functional deficits.

• **Cerebral hypoperfusion and blood-brain barrier (BBB) dysfunction:** Cerebral function depends on an adequate blood supply and an intact BBB, which is crucial for maintaining homeostasis of brain microenvironment and protecting the parenchyma from pathogens, circulating immune cells, ionic changes and toxic metabolites [121]. There is evidence of an age-related decline in cerebral microvascular structure [122] and vascular pathology has been shown to accompany age-related cognitive impairment and neurodegeneration [123]. BBB leakiness is seen both with normal ageing and AD [124,125]. In a model of AD in transgenic mice BBB permeability increase even preceded neuritic plaque formation [126] and in a neuropathological study, the ApoE4 allele, which is a major risk factor for developing AD, was associated with greater likelihood of BBB disruption [127]. Vascular cells and specifically endothelial cells and pericytes have been shown to undergo senescence in vitro and in vivo [128]. Accumulation of senescent endothelial cells is associated with impaired tight junction structure and compromised blood-brain barrier integrity [129] and it is linked to Sirt1 downregulation in senescent endothelial cells [130]. Although senescence has not been studied in the cellular components of the choroid plexus, it is known to undergo several age and disease-related structural and functional alterations [131,132]. The choroid plexus produces CSF and forms an interface between blood and CSF. It secretes trophic factors, such as epidermal growth factor (EGF) and fibroblast growth factor-2 (FGF-2) and may be a route for trafficking lymphocytes to and from the CNS with roles in immune surveillance and neuroinflammation [133,134]. In addition, a shift towards an interferon I-dependent expression profile is seen with ageing in human and mouse choroid plexus, which may adversely affect cognitive function and hippocampal neurogenesis [135]. It is conceivable that compromised cerebrovascular perfusion and altered function of the BBB and/or choroid plexus may adversely affect neuronal and glial survival.

6. Evidence of Cellular Senescence in CNS Cell Types

6.1. Astrocytes

The astrocyte is the most abundant cell type of the CNS with a prominent role in the complex functions of the healthy CNS, as well as in various pathologies [136]. Over the last few years, evidence concerning astrocyte senescence has started to emerge. It has been reported that cultured rat astrocytes show characteristics of senescence, such as increased SA-β-gal staining, robust ROS production and decreased mitochondrial activity, resulting in the loss of their ability to maintain neurons and therefore exerting detrimental effects in the aging brain [137,138].

SASP appears to be another important component of astrocyte senescence [139]. Glutathione depletion in human astrocyte cultures activated SASP-associated pathways (NF-kB and p38MAPK) and triggered secretion of IL-6 [140]. Other studies also showed that cultured astrocytes of human and rodent origin can undergo both stress-induced and replicative senescence, which is, interestingly, telomere-independent. They are characterized by an enlarged and flattened morphology and increased levels of p53, p21CIP1, p16INK4a and SA-β-gal, as well as the formation of SAHF [141,142]. Several substances and environmental toxins have been associated with astrocyte senescence. The dioxin TCDD can induce premature senescence in rodent astrocytes through activation of the WNT/β-catenin signaling pathway and ROS production and is characterized by increased levels of senescence markers, such as SA-β-gal, p16 and p21 [143]. Ammonia has also been shown to trigger cellular senescence in cultured rat astrocytes, mediated by ROS and p38MAPK activation leading to growth arrest and elevated SA-β-gal and p21 levels [144]. Paraquat can induce astrocyte senescence
and SASP in vitro, characterized by elevated levels of SA-β-gal and p16INK4a, secretion of IL-6 and increased number of 53BP1 foci [145]. These data provide a mechanistic link between environmental factors, cellular senescence and the risk of neurodegenerative disease [146].

A recent study by Crowe et al. (2016) reported that oxidative stress-induced senescence can cause several transcriptomic changes on human astrocytes. More specifically, genes associated with the development and differentiation of the nervous system, as well as cell cycle genes were downregulated, whereas genes associated with inflammation, extracellular remodeling and apoptosis resistance were upregulated [147]. Aβ has been shown to trigger astrocyte senescence with increased production of IL-6 regulated by p38MAPK [148], which corroborates a potential role of astrocytic senescence in AD pathology. In line with these results, Hou et al. also reported that SASP is expressed in senescent astrocytes and regulated by p38MAPK in a NF-κB-dependent manner [149], while Mombach et al. designed a logical model, where p38MAPK has a central role in the regulation of astrocyte senescence and SASP, in response to DNA damage [150]. Finally, prematurely aged BUBR1 mutant mice display alterations in gliosis from activated astrocytes, providing in vivo evidence of a link between accelerated cellular senescence and astrocytic dysfunction [151].

6.2. Microglia

Microglial cells are of mesenchymal origin and are the main representative of innate immune response in the CNS [152]. Microglial cells have been shown to undergo senescence with typical features. Cultured rat microglial cells have been reported to undergo replicative senescence due to telomere shortening [153] and the same finding was later reported for microglial cells from AD patients [154]. Liposaccharide treatment of BV2 microglial cells in culture led to the development of a senescence-like phenotype with growth arrest, SA-β-Gal upregulation and SAHF [155]. With ageing, microglial cells exhibit dystrophic changes, which are thought to be distinct from their typical reactive morphology. This dystrophic microglial phenotype is also associated with functional changes, it is more abundant in neurodegenerative conditions such as AD and may even precede the onset of neurodegeneration, indicating that there may be a causal relationship between microglial senescence and neurodegeneration [100,156,157]. In addition, an RNA sequencing study of age-related transcriptional changes in astrocytes revealed that in the aged mouse brain astrocytes acquire a pro-inflammatory reactive phenotype in response to induction by microglial cells. Nonetheless, this study did not examine any senescence markers that would allow us to attribute this age-related pro-inflammatory state of microglia and astrocytes to cellular senescence and SASP [158].

6.3. Oligodendrocytes

Oligodendrocytes are terminally differentiated post-mitotic cells that form the myelin sheaths of myelinated axons. They are known to be extremely vulnerable to oxidative stress [159]. Evidence of oxidative DNA damage and upregulated SA-β-Gal suggest that oligodendrocytes may undergo stress-associated cellular senescence in ageing individuals [160]. Neuroimaging and neuropathological data indicate that there is myelin damage in the white matter associated with ageing and AD [161–163], which could at least be partially explained by oligodendrocyte senescence [164].

6.4. Oligodendrocyte Progenitor Cells

Oligodendrocyte progenitor cells (OPCs) are a population of adult progenitors which constitute approximately 3–10% of glial cells [165]. Under some circumstances they are capable of undergoing asymmetric division and mediate remyelination by differentiating into myelinating oligodendrocytes, a process highly relevant to myelin repair in multiple sclerosis [113]. Although OPCs do not undergo replicative senescence [166], there is in vitro evidence that under some circumstances they enter a senescence-like state. OPC senescence is induced by the esophageal cancer-related gene 4 (Ecrg4) and is characterized by cell cycle arrest and increased expression of SA-β-Gal [167]. Interestingly, Ecrg4 exhibits increased expression in OPCs and neural stem cells (NSCs) in the aged mouse brain.
It has been noted that spontaneous remyelination in MS fails with ageing [118,168,169]. In addition, BUBR1 insufficiency, which causes a state of accelerated senescence impairs adult OPC proliferation in vivo [170]. Oligodendrocytes and oligodendrocyte precursor cells (OPCs) dysfunction, as well as myelin breakdown have been suggested to also play an important role in the pathogenesis and progression of AD, although the exact mechanisms remain unclear [164,171].

6.5. Neurons

Although neurons are post-mitotic and don’t fit the strict definition of cellular senescence, several lines of evidence suggest that even mature post-mitotic neurons develop a senescence-like phenotype. Neurons of aged mice accumulate increased amounts of double strand DNA breaks, SA-β-Gal and proinflammatory cytokines [172]. About 20–80% of mature neurons of aged mice exhibit a senescence-like phenotype with increased levels of DNA damage, heterochromatinization, SA-β-Gal activity, p38MAPK activation and production of SASP-related mediators including ROS and IL-6 [173]. Interestingly, this senescence-like phenotype was aggravated by a genetic background of dysfunctional telomeres (terc KO mice) and rescued by a CDKN1A KO background, indicating that the senescence-like phenotype is p21-mediated in aged murine neurons [173]. The demonstration of granular cytoplasmic lipofuscin deposits with ageing [174] supports the notion that human neurons may also acquire an ageing-related senescence-like phenotype. There is little data regarding the functional activity of these senescent-like neurons. Nevertheless, neurons from nuclei of the sleep-wake cycle seem to be particularly prone to lipofuscin accumulation with ageing and those lipofuscin positive neurons exhibited poorer dendritic arborization and decreased neurotransmitter production, indicative of functional compromise [175]. In addition, neurons deriving from reprogrammed fibroblasts from patients with Rett syndrome, a neurodegenerative condition due to a MECP loss of function mutation, exhibit evidence of double strand DNA damage and p53-mediated SASP, providing in vitro evidence of a link between cellular senescence and neurodegenerative disease in humans [176].

6.6. Neural Stem Cells (NSCs)

The therapeutic potential of NSCs in AD has been under thorough investigation in the last few years [177]. Meanwhile, accumulating evidence suggests that these cells are also prone to senescence. NSCs may undergo senescence in vitro in response to various stressors [178]. Specifically, after long-term incubation with Aβ oligomers, cultured NSCs have been reported to exhibit characteristics of senescence, such as enlarged and flattened morphology, increased levels of SA-β-gal and p16 and decreased level of pRb, a response mediated by the p38MAPK pathway [179,180]. These senescent NSCs have also been observed in the dentate gyrus of the APP/PS1 transgenic mouse AD model [179]. NSCs exhibit features of cellular senescence such as telomere shortening, and ROS production with ageing [181,182]. Furthermore, there is evidence from the BUBR1 KO mouse that accelerated cellular senescence impairs adult neurogenesis in vivo [110].

7. Cellular Senescence in Alzheimer’s Disease, Parkinson’s Disease and Multiple Sclerosis

7.1. Alzheimer’s Disease

Cognitive decline in AD is associated with the disseminated formation of extracellular amyloid plaques, intracellular neurofibrillary tangles comprising of hyperphosphorylated tau proteins, as well as neuronal and synaptic loss [183]. A plethora of evidence links cellular senescence with AD. Aβ42 oligomers are reported to trigger the senescent phenotype in in vitro studies with mouse neural stem cells, leading to increased numbers of SA-β-Gal positive cells [179]. Several in vivo studies in mouse models of AD corroborate these findings [179]. Increased level of SA-β-Gal was also found in plasma samples from AD patients, compared to controls [184,185]. However, SA-β-Gal was significantly decreased in monocytes and lymphocytes from AD patients compared to controls, a finding attributed to the up-regulation of miR-128 [186].
Cumulative evidence suggests that aberrant cell cycle re-entry of the terminally differentiated post-mitotic neurons may play a critical role in the pathogenesis of AD, a theory that is supported by the re-expression of several cell-cycle regulating proteins in vulnerable neurons [187–190]. More specifically, the cyclin-dependent kinase inhibitor p21CIP1, appears to be a critical mediator of cell-cycle dysregulation in AD [191]. However, the evidence remains inconclusive, as a number of studies have reported increased levels in the brains of AD patients compared to controls [192,193], while others have found no significant differences [194]. Interesting are also the results from AD and tauopathy mouse models [195,196], as well as from studies of peripheral blood lymphocytes and monocytes of AD patients [197,198]. The levels of p16INK4a have been reported to be elevated in neurons from AD patients [194,198–200], as well as in neurons from AD mouse models [195]. Increased levels of p53, a key mediator of cellular senescence and apoptosis, have been reported in different brain regions and in lymphocytes from AD patients [192,201–205], as well as in neurons of mouse models of AD [204].

Increased p38MAPK activity has been reported in AD brains and lymphocytes [205–208], as well as in the cortex of a mouse model of AD [209]. Since p38MAPK is a major regulator of SASP [209], it is not surprising that a number of key components of SASP appear to be up-regulated in AD [210,211]. Most notably, IL-6, IL-1β, TGF-β and TNF-α levels have been reported to be elevated in AD brain tissue [96,97,212–214], as well as in AD patients’ CSF and serum [215–222], while increased levels of metalloproteinases MMP-1, MMP-3 and MMP-10 have also been reported in AD [223–226].

Epigenetic modifications appear to play an important role in the pathogenesis of the disease, as differences in overall methylation have been observed in AD-affected brain regions and abnormal DNA methylation patterns have been reported in several genes associated with AD [227–229]. Moreover, elevated phosphorylated histone γH2AX (H2A histone family member X) levels have been reported in the hippocampus and lymphocytes from AD patients, indicating an active DNA damage response [230,231].

Several lines of evidence suggest that deficits in autophagy and lysosomal dysfunction contribute to the etiology and progression of neurodegenerative diseases and especially AD [232,233]. This is supported by a number of studies reporting dysregulation in many autophagic/lysosomal pathways in the context of AD [234,235], while the vast majority of AD-associated genes appears to be related to these same pathways [232]. A recent study attempted to shed light on the interplay between autophagic/lysosomal impairment and mitochondrial dysfunction and their relation to stress-induced premature senescence (SIPS) [236]. All aspects of mitochondrial function have been reported to be impaired in AD [237], including aberrant mitochondrial dynamics and structure [238] and increased oxidative stress, which is already present in the very early stage of the disease and precedes the major pathologic hallmarks, such as senile plaques and neurofibrillary tangles [132,193]. Therefore, mitochondria and lysosomes appear to have a critical role in the progression of SIPS [239], although further research is needed to elucidate their exact contribution to AD and senescence.

Besides neurons, all different cell types that are involved in AD pathology have been reported to undergo senescence. Astrocytes are key players in the initiation and progression of the disease and can have both beneficial and detrimental effects, depending on different factors [240]. Aβ oligomers can induce senescence in human astrocytes and through the activation of p38MAPK pathway lead to the production of SASP components, such as IL-6 and MMP-1 [128]. Furthermore, increased levels of γH2AX have been found in astrocytes from AD hippocampal samples [241]. Microglia has long been implicated in the pathogenesis of AD, although the exact underlying mechanisms remain elusive [242,243]. Cultured microglial cells from AD patients have been reported to undergo replicative senescence due to telomere shortening [153]. Moreover, neuropathological features of AD have been associated with dystrophic microglial cells that exhibit morphological changes indicative of senescence [157]. A recent study reported that in vitro aged microglia from rats [244], after treatment with Aβ oligomers acquire a senescent phenotype, characterized by increased levels of SA-β-gal, IL-1β, TNF-α and MMP-2 [245]. Finally, an association between telomere shortening and AD has been
suggested [246–248]. However, a large community-based longitudinal study reported no difference in the telomere length between incident pure AD patients and cognitively healthy individuals [249]. More studies are needed to shed light on the plausible connection between telomere length and AD.

7.2. Parkinson’s Disease

PD pathology is mainly characterized by loss of neurons from the substantia nigra pars compacta in association with the accumulation of ubiquitinated alpha synuclein and other proteins in cytoplasmic inclusions (Lewy bodies) and thread-like proteinaceous inclusions within neurites (Lewy neuritis). However, Lewy bodies are also seen in the cerebral cortex, brainstem nuclei, limbic system, sympathetic ganglia, nucleus basalis of Meynert and myenteric plexus [4]. A great deal of data supports a role of cellular senescence in the pathogenesis of PD. The expression of cell-cycle genes has been found upregulated in PD. Specifically, p16\textsuperscript{INK4a} mRNA levels were elevated in PD brain samples compared to controls [145]. Increased pRb, another important regulator of cell-cycle progression, was reported in the cytoplasm of neurons in the substantia nigra of PD patients compared to age-matched controls [234]. The same study showed that the serine 795 phosphorylated, inactive form of pRb (ppRb), had a distinct distribution pattern in PD cases [250]. Another study reported increased levels of the E2F-1 transcription factor in dopaminergic neurons in the substantia nigra of PD patients and suggested that the pRb/E2F-1 pathway is activated in these neurons which can lead to apoptosis [251]. Increased levels of SA-β-gal have also been found in the CSF from PD patients compared to healthy controls [252].

Several SASP-related factors have been found upregulated in PD. IL-1β levels have been reported to be elevated in the CSF [218], serum [222] and dopaminergic regions of the striatum from patients with PD compared to controls [253]. Increased levels of IL-6 in PD patients’ serum have been reported in a number of studies [254–256], while IL-6 levels have also been associated with disease severity [257]. IL-6 levels have also been found elevated in the striatal dopaminergic region [253], as well as the CSF from PD patients compared to controls [218]. Elevated TNF-α levels have been reported in the striatum and the CSF of PD patients compared to controls [258], while MMP-3 was found to co-localize with α-synuclein in the Lewy bodies in PD patients’ brains [259]. However, it is not clear whether the elevated levels of these cytokines can be attributed to SASP in PD or they are merely part of a separate neuroinflammatory process, which is an established part of the pathophysiology of the disease [260].

Several lines of evidence indicate that mitochondrial dysfunction plays a central role in the pathophysiology of PD. Different mutations in the genes involved in familial PD are associated with pathways of mitochondrial dysfunction, while some of these compromised pathways have been established as important factors in the pathophysiology of sporadic PD [261]. Autophagic/lysosomal dysfunction are also thought to have a key role in the pathogenesis of the disease, with many PD mutations being associated with defects in these pathways [262,263]. Interestingly, a number of key mutations are involved both in mitochondrial and autophagic/lysosomal dysfunction pathways, revealing a compelling crosstalk that lies in the center of the pathophysiology of PD [264].

A recent study by Chinta et al. found increased numbers of senescent astrocytes in substantia nigra tissue samples from PD patients, as compared to controls [145]. The same study also reported that paraquat, an herbicide that has been strongly associated with the development of sporadic PD, was able to induce senescence in human astrocytes [145].

The evidence concerning telomere length in PD remain inconclusive, [246] with a number of different studies reporting contradictory results [265–270]. A meta-analysis by Forero et al., incorporating all these studies, showed that there is no difference in telomere length between PD patients and age-matched controls [270].

7.3. Multiple Sclerosis

Multiple sclerosis (MS) is a chronic, immune mediated disease characterized by inflammatory demyelination, astrogliosis, neuronal and axonal loss involving the brain and spinal cord [271]. Its aetiology remains unclear but genetic and environmental factors are thought to influence the
likelihood of developing the disease [272]. The majority of MS patients follow an initial course with relapses followed by some degree of remission called relapsing-remitting MS (RR-MS). Relapses in RR-MS are driven by inflammation which can be visualized as new focal inflammatory demyelinating lesions with magnetic resonance imaging (MRI) techniques. Several immunomodulatory and immunosuppressive disease-modifying treatments are currently available with moderate to high efficacy in tackling inflammation in RR-MS [273]. Nevertheless, after variable time RR-MS gradually transforms into secondary progressive MS (SP-MS), a phase with progressive build-up of disability. In the secondary progressive phase of MS (SP-MS) new focal inflammatory demyelinating lesion formation is rare, and the pathological correlate of disability progression is neuroaxonal loss driven by neurodegenerative mechanisms [274–276]. The pathogenesis of ongoing neuroaxonal loss and the time of shifting from the relapsing to the secondary progressive phase of the disease are poorly understood. Epidemiological evidence suggests that age is the most important determinant for the transition to the progressive phase of MS [11]. Several immunomodulatory and immunosuppressive therapies have failed in the progressive forms of MS. Licensed therapeutic options for preventing disease progression in SP-MS are lacking.

Oxidative damage and mitochondrial dysfunction are key features of MS pathology [277–280]. Cellular senescence is an age-dependent process known to be accelerated by oxidative stress and chronic inflammation [14,20]. Random irreparable ROS-mediated damage to the DNA of cells and mitochondrial dysfunction are strong inducers of cellular senescence [14]. We postulate that accelerated accumulation of senescent cells above a certain threshold may determine the shift to the secondary progressive phase of MS and that neurodegeneration in progressive MS is driven by senescence-associated loss of function. Furthermore, the so-called “compartmentalized within the blood-brain barrier (BBB)” inflammation [276], which is resistant to our immunomodulatory strategies, may represent the SASP-associated low burning inflammation. In line with our hypothesis, currently used immunomodulatory and immunosuppressive treatments are modestly or not effective in the secondary progressive phase of the disease but may delay the onset of the secondary progressive phase when used early in the inflammatory relapsing phase [281], probably due to their efficacy in preventing new inflammatory demyelinating lesion formation and the oxidative DNA damage associated with it.

In the cuprizone-induced demyelination model of multiple sclerosis, increased numbers of senescence-associated β-galactosidase positive senescent glial cells were detected in the chronically demyelinated corpus callosum. This finding was confirmed with GL13 lipofuscin histochemistry. Correlation analysis revealed a significant association between the number of senescent cells and the extent of demyelination and motor performance, indicating a link between chronic demyelination and senescent glial cell load and between the senescent glial cell load and loss of function [282]. Using GL13 histochemistry as a marker for cellular senescence we detected lipofuscin+ glial cells in acute active (Figure 1A iv) and chronic active demyelinated white matter lesions from SP-MS cases (Figure 1B iv). Lipofuscin positive senescent cells were sparse in chronic inactive demyelinated lesions (Figure 1C iv). No lipofuscin+ glial cells were detected in the normal appearing white matter (NAWM) (data not shown). Cortical demyelination is common and extensive, particularly in the progressive stages of MS [283]. The extent of cortical demyelination has been shown to correlate with disability, cognitive impairment and the likelihood of developing seizures [284]. The most abundant type is the subpial cortical demyelinated lesion which extends from the pial surface into the deeper cortical layers. A gradient of neuronal loss greater at the most superficial layers I and II and lesser at deeper layers V and VI has been described [285]. Yet, cortical neuronal and synaptic loss have been demonstrated in the absence of demyelination [286]. GL13 histochemistry of subpial demyelinated cortical lesions (Figure 1D iv) and normal appearing cortex revealed granular lipofuscin deposits in numerous neurons (Figure 1E iv), indicating that neurons in SP-MS exhibit a senescence-like phenotype. Evidence of lipofuscin accumulation in glial cells and neurons in grey and white matter demyelinated lesions in SP-MS corroborate the hypothesis of cellular senescence playing a pathogenetic role in progressive MS. Nevertheless, these findings merit further quantitative investigation in order to differentiate the effects
of ageing from those of MS and to potentially associate the extent of cellular senescence with other pathological features and clinical parameters.

**Figure 1.** Lipofuscin accumulation as a marker of cellular senescence in multiple sclerosis lesions. Demyelinated lesions were identified with myelin basic protein (MBP) immunohistochemistry and were staged according to Trapp et al. (1998) [287] as acute active, chronic active or chronic inactive using human leukocyte antigen-DR isotype (HLA-DR) immunohistochemistry on serial sections from paraffin embedded postmortem tissue blocks. Lipofuscin was detected with the GL13 hybrid histochemistry-immunohistochemistry method [58]. Acute active demyelinated white matter lesion with MBP staining showing ongoing perivascular demyelination in subcortical white matter from the parietal lobe of a 73-year-old secondary progressive multiple sclerosis (SP-MS) patient (MS51) (A(i)). Infiltration with HLA-DR+ cells with macrophage morphology throughout the demyelinated parenchyma (HLA DR immunohistochemistry) (A(ii)). Perivascular infiltration with CD8+ lymphocytes (CD8 immunohistochemistry) (A(iii)). GL13 staining in acute active lesions showed lipofuscin+ cells. Although many of them were perivascularly localized, some were not, suggesting that at least some of them maybe glial cells rather than inflammatory cells (A(iv)). Chronic actively demyelinating perivenentricular white matter lesion with a fully demyelinated lesion center (lack of MBP immunoreactivity) from a 74-year-old female MS patient (MS265) (B(i)). Typically, HLA-DR immunohistochemistry of serial sections exhibited a border infiltrated by numerous macrophages...
whereas the lesion centre is infiltrated by ramified microglia (B(ii)). Few CD8$^+$ lymphocytes are present perivascularly (CD8 immunohistochemistry) (B(iii)). Lipofuscin$^+$ cells with granular staining were found in the macrophage infiltrated lesion border (B(iv)). Chronic inactive subcortical white matter demyelinated lesion (lack of MBP immunoreactivity with a well demarcated border) from the left parietal lobe of a 71-year-old female SP-MS patient (MS33) (C(i)). Ramified microglial morphology throughout the demyelinated lesion area and lesion border (HLA-DR immunohistochemistry) typical of a chronic inactive lesion (C(ii)). Decreased axonal density in the demyelinated lesion seen with 200 kDa neurofilament immunohistochemistry (C(iii)). Numerous parenchymal lipofuscin$^+$ cells in the demyelinated white matter. Lack of HLA-DR$^+$ macrophages from the chronic demyelinated lesion suggests that the lipofuscin$^+$ cells are glial (C(iv)). Subpial cortical demyelination (lack of MBP immunoreactivity extending from the pial surface into the deeper cortical layers from the parietal cortex of a 71-year-old female SP-MS patient (MS33) (D(i)). HLA-DR$^+$ ramified microglia in the demyelinated cortical lesion (D(ii)) and few CD8$^+$ lymphocytes infiltrating the adjacent pia matter (D(iii)). Lipofuscin$^+$ cells mostly with neuronal morphology (inset) throughout the demyelinated cortex (D(iv)). Normal appearing cortex with intact appearing cortical myelin (MBP immunohistochemistry) from the left parietal lobe of a 71-year-old female SP-MS patient (MS33) (E(i)), HLA-DR immunoreactivity revealing quiescent ramified microglia (E(ii)) and normal-appearing axonal staining with 200 kDa neurofilament immunohistochemistry on a serial section (E(iii)). GL13 staining showed numerous lipofuscin$^+$ cells mostly with neuronal morphology (E(iv)). Scale bars represent 500 µm (A(i),A(ii),B(i),B(ii),C(i),C(ii),C(iii),D(i),D(ii),E(i),E(ii)), 50 µm (D(iii),E(iii)) or 25 µm (A(iii),A(iv),B(iii),B(iv),C(iv),D(iv),E(iv)).

8. Cellular Senescence as a Therapeutic Target

Currently, there are no available neuroprotective treatments that can effectively modify the disease course and prevent disease progression for AD or PD. Several attempts at targeting Aβ amyloid in AD have failed [288,289]. Interestingly, the Aβ plaques may be found in 35% of cognitively healthy individuals above the age of 60 [290] and the Aβ load, which has been our main target, correlates better with age than disease severity in AD [291–293], casting doubt on the amyloid hypothesis. Similarly, therapies in PD aim to substitute dopamine and restore the dopaminergic system deficit, with no effect on neuronal cell loss and consequently on disease progression. In MS only siponimod, an S1P1/S1P3 receptor modulator, which is not currently licensed, has shown a modest effect in a phase III trial (EXPAND) in secondary- progressive MS, preventing disability progression by 21% in two years [294]. Therefore, there is an urgent need for neuroprotective treatments for neurodegenerative disease. Investigation into new treatment approaches may require a paradigm shift in our view of the pathogenetic mechanisms of neurodegeneration.

Several lines of evidence implicate cellular senescence in the pathogenesis of neurodegenerative disease. Targeting cellular senescence as a therapeutic strategy is promising yet still at an embryonic stage. There is evidence of a beneficial effect of both pro-senescent and anti-senescent approaches, depending on context. A pro-senescent effect may be desirable in treating cancer [295–297], renal, liver and cutaneous fibrosis [298–301]. An anti-senescent treatment approach may be beneficial in neurodegenerative disease. An anti-senescent or senotherapeutic approach may involve the selective death of senescent cells in order to reduce the burden of senescent cells on a tissue (senolysis) or modulate senescent cells (senomorphism) in a way that neutralizes the detrimental effects of senescent cells in a tissue i.e., by blocking the expression of SASP or particular mediators of SASP.

Senolysis and/or senomorphosis in neurodegenerative disease would aim at preventing cell loss and tissue destruction in order to ultimately prevent disease progression. Given that neurodegenerative diseases seem to have a long presymptomatic phase with pathological changes appearing several years before clinical presentation e.g., 50–60% of nigral neurons are already lost at PD diagnosis [302], a great effort is being made to diagnose neurodegenerative disease presymptomatically using different biomarkers. In the context of presymptomatic diagnosis, early senotherapeutic treatment could, in theory, even prevent the clinical presentation of neurodegenerative disease. So far, the most
convincing senotherapeutic manipulation comes from a sophisticated experiment in genetically modified BubR1 progeroid mice. In this setting, \( \text{p16}^{\text{INK4A}} \) senescent cells were eliminated by activation of an INK-ATTAC transgene by drug treatment. Lifelong elimination of \( \text{p16}^{\text{INK4A}} \) cells substantially delayed age-related disease, whereas late life elimination of \( \text{p16}^{\text{INK4A}} \) cells attenuated these age-related pathologies [38], supporting the notion that cellular senescence can be successfully exploited therapeutically.

A number of compounds with senolytic or senomorphic actions have been examined in vitro and in vivo with notable results, summarized in Table 1. From our limited experience so far it is evident that, in most cases, the senescence-modifying action is not universal, but rather cell-type dependent, which greatly complicates the therapeutic landscape [303]. Many of the promising compounds with senolytic or senomorphic activity such as metformin or dasatinib are in use with different indications (diabetes mellitus type 2 and CML/ALL, respectively), which suggests that drug repurposing may facilitate our quest for efficacious senotherapeutics. Nevertheless, senotherapeutics have not been examined in models of neurodegeneration and supportive evidence remains weak and indirect, and sometimes even contradictory. For example, although some epidemiological studies supported a protective role for metformin, which crosses the BBB, in preventing cognitive decline in individuals with type 2 diabetes [304], another 12-year cohort study in patients with type 2 diabetes showed a two-fold increase in the risk of AD and PD in those who took metformin compared to those who didn’t [305]. The complex physiological and pathophysiological roles of cellular senescence, exerting both beneficial and detrimental effects according to setting, along with the cell-type specific variability in senescence triggers and senescence phenotypes, necessitates a cautious approach to avoid pitfalls when targeting a such a key biological process therapeutically. Furthermore, the relationship between senescence and immune response merits further elucidation. Naturally occurring immune-mediated clearance of senescent cells could be exploited therapeutically by developing medications that enhance it. The high specificity of immune responses could be employed to specifically target senescent cell types by developing senolytic vaccines. Cell surface markers of senescence [306] or even the intracellularly localized lipofuscin could potentially be used to prime the cellular or humoral immune response, directing it against senescent cells. Expansion of our knowledge of cellular senescence and its extensive investigation in numerous settings is warranted.
Table 1. Licensed and experimental compounds with senolytic, senomorphic or senescence-inducing action adapted from Myrianthopoulos et al., 2018 [303]. ALL: acute lymphocytic leukemia, CML: chronic myelogenous leukemia, HUVEC: human umbilical vein epithelial cells, JAK: Janus kinase, MEF: mouse embryonic fibroblasts, MoA: mechanism of action, PPI: protein-protein interaction inhibitor.

| Compound          | MoA                                                                 | Effect                                                                 | Current Indication                                      | Classification | References          |
|-------------------|----------------------------------------------------------------------|------------------------------------------------------------------------|---------------------------------------------------------|----------------|---------------------|
| Dasatinib (Sprycel) | Tyrosine kinase inhibitor, Inhibitor of Eph receptors                | Reduced proliferation of senescent cells in vitro; Alleviated ageing phenotypes in treated animals | Philadelphia chromosome-CML and ALL                    | senolytic      | [307–310]          |
| Quercetin         | Modulator of NF-κB, PI3K/Akt, estrogen receptor, mTOR, PIKδ kinase inhibitor, Potent antioxidant | Kills senescent human endothelial cells and murine bone marrow mesenchymal stem cells | experimental senolytic |                | [311–323]          |
| Navitoclax (ABT-263) | BCL-2 inhibitor (PPI)                                                | Reduced survival of HUVEC, human lung fibroblasts and murine embryonic fibroblasts and mesenchymal stem cells in vitro | experimental Senolytic |                | [324–330]          |
| ABT-737           | BCL-XL inhibitor (PPI)                                               | Reduced viability of senescent cell in vitro and in vivo               | experimental senolytic |                | [331–334]          |
| A1331852 and A1155463 | BCL-XL inhibitor                                                   | Reduced viability of senescent cell in vitro.                           | experimental senolytic |                | [335]               |
| Fisetin           | Interacts with: topoisomerases, cyclin-dependent kinases, NF-κB, PPAR, PARP1, PI3K/Akt/mTOR, antioxidant | Delay of age-related CNS complications in vivo                          | experimental senomorphics                             |                | [335–346]          |
| Piperlongumine    | NF-κB modulator                                                     | Induces apoptosis in aged cells                                         | experimental senolytic |                | [347,348]          |
| Geldanamycin      | HSP90 inhibitor, down-regulation of PI3K/Akt                         | Induces death of senescent cells in vitro                              | experimental senolytic |                | [349,350]          |
| Tanespimycin (17-AAG) | HSP90 inhibitor, down-regulation of PI3K/Akt                      | Induces death of senescent cells in vitro                              | experimental senolytic |                | [349,350]          |
| Panobinostat (Farydak) | non-selective histone deacetylase inhibitor                        | Synergistic effect with taxol in inducing death of senescent cells, in vivo. | multiple myeloma senolytic |                | [351]               |
| Apigenin and Kaempferol | Interference with the NF-κB p65 subunit and IκB                      | Inhibited components of SASP such as in IL-6, CXCL and GM-CSF in vivo. | experimental senomorphics                             |                | [352]               |
| Rapamycin (Rapamune) | mTOR kinase inhibitor                                               | suppression of replicative senescence of rodent embryonic cells, lifespan extension in model systems | Lymphangio-lymphangiomatosis, coronary stent clot prevention, prevention of transplant rejection | senomorphic    | [353–356]          |
| Ruxolitinib (Jakafi) | JAK inhibitor                                                      | decreased systemic inflammation in aged animals and improved age-related dysfunctions and alleviating frailty | Polycytemia vera, Myelofibrosis senomorphic             |                | [357]               |
Table 1. Cont.

| Compound              | MoA                                      | Effect                                           | Current Indication                  | Classification                  | References |
|-----------------------|------------------------------------------|--------------------------------------------------|-------------------------------------|---------------------------------|------------|
| Metformin (Glucophage™) | Inhibition of phosphorylation of IkB kinase | Increases lifespan, inhibits SASP, Prevents senescence in a disk degeneration model | Diabetes mellitus type 2           | senolytic                       | [358–361] |
| Cortisol and corticosterone |                                           | Prevented senescence of human fibroblasts in vitro | Inflammation, allergy              | senomorphic                     | [362]     |
| Resveratrol and derivatives | SIRT1 and IkB inhibitor | Attenuates SASP in human fibroblasts in vitro | Dietary supplement                 | Senomorphic/ senolytic/senescence modulator | [363–365] |
| Loperamide            | Opioid receptor agonist and Ca++ channel blocker | Prevented senescence of primary MEFs in vitro | antidiarrheal                      | senomorphic                     | [349]     |
| Niguldipine           | Ca++ channel blocker, a1 adrenoreceptor antagonist | Prevented senescence of primary MEFs in vitro | experimental                       | senomorphic                     | [349]     |
| Nutlin3a              | p53 stabilizer                           | Prevented or inverted pulmonary hypertension in an in vivo model | experimental                       | Inducer of senescence           | [366–368] |
| Dexamethasone         | SIRT1 inhibition and p53/p21WAF/CIP1 activation | Increased percentage of senescent tenocytes in vitro and in vivo | Anti-edematous, anti-inflammatory | Inducer of senescence           | [369]     |
9. Conclusions and Future Perspectives

Senescent cells accumulate with ageing and progeroid models have provided in vivo experimental data of accelerated ageing-related degenerative pathologies. In addition, experimental senolysis ameliorated ageing-related pathologies [38]. Thus, cellular senescence meets the criteria for a potentially causal role in ageing-related disease. There is evidence of cellular senescence affecting astrocytes, microglia, oligodendrocyte progenitors and neural stem cells. A senescence-like phenotype has also been demonstrated in post-mitotic cells, which suggests that neurons and oligodendrocytes may also become senescent. Resident brain cells are either post-mitotic or slowly cycling. They are more likely to exhibit stress-induced premature senescence due to various stressors or insults than develop replicative senescence. However, evidence connecting cellular senescence with the mechanisms of neurodegeneration remains indirect and further investigation of the putative role of senescence in neurodegeneration is required. Unequivocal identification of senescent cells in vitro and in vivo is an important prerequisite to facilitate our understanding of cellular senescence and its role in different cell types. Detecting lipofuscin as a marker of cellular senescence using the GL13 compound, which not only detects lipofuscin in situ but also in biological fluids [93], is likely to boost our understanding of the senescence process. Casting light on CNS cell senescence and its role in neurodegeneration is essential to inform any practices that may be senescence-inducing e.g., using corticosteroids [368], beta-interferons [370] or DNA-damaging chemotherapeutics in MS, practices that may prove detrimental in the long run. Secondly, there is an urgent need for disease-modifying cures for neurodegenerative diseases. Cellular senescence may be a credible therapeutic target opening new therapeutic avenues for neurodegenerative disease and senotherapeutics may prove to be efficacious neuroprotectants.

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Abbreviations

Aβ amyloid-beta
AD Alzheimer’s disease
Akt protein kinase B
ALL acute lymphocytic leukemia
APP amyloid beta precursor protein
ATF6α activating transcription factor 6 isoform α
ATM ataxia-telangiectasia mutated
BBB blood-brain barrier
Bcl-2 B-cell lymphoma 2
CDK cyclin-dependent kinase
CDKI cyclin-dependent kinase inhibitor protein
cGAS cyclic GMP-AMP synthase
CIP/KIP CDK interacting protein/kinase inhibitory protein
CML chronic myelogenous leukemia
CNS central nervous system
CSF cerebrospinal fluid
DDR DNA damage response
Ecrγ4 esophageal cancer-related gene 4
EGF epidermal growth factor
ER endoplasmic reticulum
FGF-2 fibroblast growth factor 2
GLB1 galactosidase beta 1
HLA-DR human leukocyte antigen-DR isotype
γH2AX phosphorylated H2A histone family member X
IkB inhibitor of kappa B
IL Interleukin
MBP myelin basic protein
MECP methyl-CpG-binding protein
MMP matrix metalloproteinase
MRI magnetic resonance imaging
mTOR mammalian target of rapamycin
MS multiple sclerosis
NAWM normal appearing white matter
NF-κB nuclear factor kappa-light-chain-enhancer of activated B cells
NSCs neural stem cells
OIS oncogene-induced senescence
OPC oligodendrocyte progenitor cell
PD Parkinson’s disease
PS1 presenilin-1
p38MAPK p38 mitogen-activated protein kinases
RB Retinoblastoma
ROS reactive oxygen species
RR-MS relapsing-remitting multiple sclerosis
SA-β-Gal senescence-associated β-galactosidase
SAHF senescence-associated heterochromatin formation
SAMD senescence-associated mitochondrial dysfunction
SASP senescence-associated secretory phenotype
SIPS stress-induced premature senescence
SIRT Siruatin
SP-MS secondary progressive multiple sclerosis
SGZ subgranular zone
STING stimulator of IFN genes
SVZ subventricular zone
SIP sphingosine 1-phosphate receptor
TCDD 2,3,7,8-Tetrachlorodibenzo-p-Dioxin
Terc human telomerase gene
TGF-β transforming growth factor beta
TNF-α tumour necrosis factor alpha
UPR unfolded protein response
53BP1 p53-binding protein 1

References
1. Johnson, I.P. Age-related neurodegenerative disease research needs aging models. *Front. Aging Neurosci.* 2015, 7, 168. [CrossRef] [PubMed]
2. Schrijvers, E.M.; Verhaaren, B.F.; Koudstaal, P.J.; Hofman, A.; Ikram, M.A.; Breteler, M.M. Is dementia incidence declining? Trends in dementia incidence since 1990 in the Rotterdam Study. *Neurology* 2012, 78, 1456–1463. [CrossRef] [PubMed]
3. Rocca, W.A.; Petersen, R.C.; Knopman, D.S.; Hebert, L.E.; Evans, D.A.; Hall, K.S.; Gao, S.; Unverzagt, F.W.; Langa, K.M.; Larson, E.B.; et al. Trends in the incidence and prevalence of Alzheimer’s disease, dementia, and cognitive impairment in the United States. *Alzheimers Dement.* 2011, 7, 80–93. [CrossRef] [PubMed]
4. Nussbaum, R.L.; Ellis, C.E. Alzheimer’s Disease and Parkinson’s Disease. *N. Engl. J. Med.* 2003, 348, 1356–1364. [CrossRef] [PubMed]
5. Lockrow, J.P.; Fortress, A.M.; Granholm, A.C.E. Age-related neurodegeneration and memory loss in down syndrome. *Curr. Gerontol. Geriatr. Res.* 2012, 2012, 13. [CrossRef] [PubMed]

6. Takeda, T.; Hosokawa, M.; Takeshita, S.; Irino, M.; Higuchi, K.; Matsushita, T.; Tomita, Y.; Yasuhira, K.; Hamamoto, H.; Shimizu, K.; et al. A new murine model of accelerated senescence. *Mech. Ageing Dev.* 1981, 17, 183–194. [CrossRef]

7. Morley, J.E.; Kumar, V.B.; Bernardo, A.E.; Farr, S.A.; Uezu, K.; Tumosa, N.; Flood, J.F. Beta-amyloid precursor polypeptide in SAMP8 mice affects learning and memory. *Peptides* 2000, 21, 1761–1767. [CrossRef]

8. Blin, P.; Dureau-Pournin, C.; Foubert-Samier, A.; Grolleau, A.; Corbillon, E.; Jové, J.; Lassalle, R.; Robinson, P.; Pouthiat, N.; Droz-Perroteau, C.; Moore, N. Parkinson’s disease incidence and prevalence assessment in France using the national healthcare insurance database. *Eur. J. Neurol.* 2015, 22, 464–471. [CrossRef] [PubMed]

9. Duncan, G.W.; Khoo, T.K.; Coleman, S.Y.; Brayne, C.; Yarnall, A.J.; O’Brien, J.T.; Barker, R.A.; Burn, D.J. The incidence of Parkinson’s disease in the North-East of England. *Age Ageing* 2014, 43, 257–263. [CrossRef] [PubMed]

10. Trapp, B.D.; Nave, K.-A. Multiple Sclerosis: An Immune or Neurodegenerative Disorder? *Annu. Rev. Neurosci.* 2008, 31, 247–269. [CrossRef] [PubMed]

11. Scalfari, A.; Neuhaus, A.; Daumer, M.; Ebers, G.C.; Muraro, P.A. Age and disability accumulation in multiple sclerosis. *Neurology* 2011, 77, 1246–1252. [CrossRef] [PubMed]

12. Kirkwood, T.B.L.; Melov, S. On the Programmed/Non-Programmed Nature of Ageing within the Life History. *Curr. Biol.* 2011, 21, R701–R707. [CrossRef] [PubMed]

13. Kirkwood, T.B.L.; Feder, M.; Finch, C.E.; Franceschi, C.; Globerson, A.; Klingenberg, C.P.; LaMarco, K.; Omholt, S.; Westendorp, R.G. What accounts for the wide variation in life span of genetically identical organisms reared in a constant environment? *Mech. Ageing Dev.* 2005, 126, 439–443. [CrossRef] [PubMed]

14. López-Otin, C.; Blasco, M.A.; Partridge, L.; Serrano, M.; Kroemer, G. The Hallmarks of Aging. *Cell* 2013, 153, 1194–1217. [CrossRef] [PubMed]

15. Muñoz-Espín, D.; Serrano, M. Cellular senescence: From physiology to pathology. *Nat. Rev. Mol. Cell Biol.* 2014, 15, 482–496. [CrossRef] [PubMed]

16. Barbouti, A.; Evangelou, K.; Pateras, I.S.; Papoudou-Bai, A.; Patereli, A.; Stefanaki, K.; Rontogianni, D.; Muñoz-Espín, D.; Kanavaros, P.; Gorgoulis, V.G. In situ evidence of cellular senescence in Thymic Epithelial Cells (TECs) during human thymic involution. *Mech. Ageing Dev.* 2018. [CrossRef] [PubMed]

17. Chuprin, A.; Gal, H.; Biron-Shental, T.; Biran, A.; Amiel, A.; Rozenblatt, S.; Krizhanovsky, V. Cell fusion induced by ERVWE1 or measles virus causes cellular senescence. *Genes Dev.* 2013, 27, 2356–2366. [CrossRef] [PubMed]

18. Demaria, M.; Ohtani, N.; Youssef, S.A.; Rodier, F.; Toussaint, W.; Mitchell, J.R.; Laberge, R.-M.; Vijg, J.; Van Steeg, H.; D’Ovidio, M.; et al. An Essential Role for Senescent Cells in Optimal Wound Healing through Secretion of PDGF-AA. *Dev. Cell* 2014, 31, 722–733. [CrossRef] [PubMed]

19. Rajagopalan, S.; Long, E.O. Cellular senescence induced by CD158d reprograms natural killer cells to promote vascular remodeling. *Proc. Natl. Acad. Sci. USA* 2012, 109, 20596–20601. [CrossRef] [PubMed]

20. Howcroft, T.K.; Campisi, J.; Louis, G.B.; Smith, M.T.; Wise, B.; Wyss-Coray, T.; Augustine, A.D.; McElhaney, J.E.; Kohanski, R.; Sierra, F. The role of inflammation in age-related disease. *Aging (Albany NY)* 2013, 5, 84–93. [CrossRef] [PubMed]

21. Hayflick, L.; Moorhead, PS. The serial cultivation of human diploid strains. *Exp. Cell Res.* 1961, 25, 585–621. [CrossRef]

22. Gorgoulis, V.G.; Halazonetis, T.D. Oncogene-induced senescence: The bright and dark side of the response. *Curr. Opin. Cell Biol.* 2010, 22, 816–827. [CrossRef] [PubMed]

23. Hernandez-Segura, A.; Nehme, J.; Demaria, M. Hallmarks of Cellular Senescence. *Trends Cell Biol.* 2018, 28, 436–453. [CrossRef] [PubMed]

24. Sabin, R.J.; Anderson, R.M. Cellular Senescence—Its role in cancer and the response to ionizing radiation. *Genome Integr.* 2011, 2, 7. [CrossRef] [PubMed]

25. Dörr, J.R.; Yu, Y.; Milanovic, M.; Beuster, G.; Kasahara, D.; Dähnrich, J.H.M.; Lisec, J.; Lenze, D.; Gerhardt, A.; Schleicher, K.; et al. Synthetic lethal metabolic targeting of cellular senescence in cancer therapy. *Nature* 2013, 501, 421–425. [CrossRef] [PubMed]
26. Toussaint, O.; Medrano, E.E.; von Zglinicki, T. Cellular and molecular mechanisms of stress-induced premature senescence (SIPS) of human diploid fibroblasts and melanocytes. *Exp. Gerontol.* **2000**, *35*, 927–945. [CrossRef]

27. Kang, H.T.; Lee, K.B.; Kim, S.Y.; Choi, H.R.; Park, S.C. Autophagy impairment induces premature senescence in primary human fibroblasts. *PLoS ONE* **2011**, *6*, e23367. [CrossRef] [PubMed]

28. Wiley, C.D.; Flynn, J.M.; Morrissey, C.; Lebofsky, R.; Shuga, J.; Dong, X.; Unger, M.A.; Vijg, J.; Melov, S.; Campisi, J. Analysis of individual cells identifies cell-to-cell variability following induction of cellular senescence. *Aging Cell* **2017**, *16*, 1043–1050. [CrossRef] [PubMed]

29. Nakamura, A.J.; Chiang, Y.J.; Hathcock, K.S.; Horikawa, I.; Sedelnikova, O.A.; Hodes, R.J.; Bonner, W.M. Both telomeric and non-telomeric DNA damage are determinants of mammalian cellular senescence. *Epigenet. Chromatin* **2008**, *1*, 6. [CrossRef] [PubMed]

30. Rodier, F.; Campisi, J. Four faces of cellular senescence. *J. Cell Biol.* **2011**, *192*, 547–556. [CrossRef] [PubMed]

31. Ramireza, R.D.; Morales, C.P.; Herbert, B.S.; Rohde, J.M.; Passons, C.; Shay, J.W.; Wright, W.E. Putative telomere-independent mechanisms of replicative aging reflect inadequate growth conditions. *Genes Dev.* **2001**, *15*, 398–403. [CrossRef] [PubMed]

32. Rodier, F.; Coppé, J.-P.; Patil, C.K.; Hoeijmakers, W.A.M.; Muñoz, D.P.; Raza, S.R.; Freund, A.; Campeau, E.; Davalos, A.R.; Campisi, J. Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. *Nat. Cell Biol.* **2009**, *11*, 973–979. [CrossRef] [PubMed]

33. Turenne, G.A.; Paul, P.; Lafllair, L.; Price, B.D. Activation of p53 transcriptional activity requires ATM’s kinase domain and multiple N-terminal serine residues of p53. *Oncogene* **2001**, *20*, 5100–5110. [CrossRef] [PubMed]

34. Xue, W.; Zender, L.; Miething, C.; Dickins, R.A.; Hernando, E.; Krizhanovsky, V.; Cordon-Cardo, C.; Lowe, S.W. Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. *Nature* **2007**, *445*, 656–660. [CrossRef] [PubMed]

35. Hoenicke, L.; Zender, L. Immune surveillance of senescent cells—Biological significance in cancer- and non-cancer pathologies. *Carcinogenesis* **2012**, *33*, 1123–1126. [CrossRef] [PubMed]

36. Herbig, U.; Jobling, W.A.; Chen, B.P.; Chen, D.J.; Sedivy, J.M. Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21CIP1, but not p16INK4a. *Mol. Cell* **2004**, *14*, 501–513. [CrossRef]

37. Lawless, C.; Wang, C.; Jurk, D.; Merz, A.; Zglinicki, T. von; Passos, J.F. Quantitative assessment of markers for cell senescence. *Exp. Gerontol.* **2010**, *45*, 772–778. [CrossRef] [PubMed]

38. Baker, D.J.; Wyshake, T.; Tckhonia, T.; LeBrasseur, N.K.; Childs, B.G.; van de Sluis, B.; Kirkland, J.L.; van Deursen, J.M. Clearance of p16Ink4a -positive senescent cells delays ageing- associated disorders. *Nature* **2012**, *479*, 232–236. [CrossRef] [PubMed]

39. Hoenicke, L.; Zender, L. Immune surveillance of senescent cells—Biological significance in cancer- and non-cancer pathologies. *Carcinogenesis* **2012**, *33*, 1123–1126. [CrossRef] [PubMed]

40. Stein, G.H.; Drullinger, L.F.; Soulard, A.; Dulić, V. Differential Roles for Cyclin-Dependent Kinase Inhibitors in Mammalian Senescence. *Mol. Cell* **1999**, *19*, 2109–2117. [CrossRef] [PubMed]

41. Sharma, V.; Gilhotra, R.; Dhingra, D.; Gilhotra, N. Possible underlying influence of p38MAPK and NF-κB in the diminished anti-effector activity of dizeepam in stressed mice. *J. Pharmacol. Sci.* **2011**, *116*, 257–263. [CrossRef] [PubMed]

42. Yang, H.; Wang, H.; Ren, J.; Chen, Q.; Chen, Z.J. cGAS is essential for cellular senescence. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, E4612–E4620. [CrossRef] [PubMed]

43. Coppé, J.-P.; Desprez, P.-Y.; Krtolica, A.; Campisi, J. The Senescence-Associated Secretory Phenotype: The Dark Side of Tumor Suppression. *Ann. Rev. Pathol. Mech. Dis.* **2010**, *5*, 99–118. [CrossRef] [PubMed]

44. Acosta, J.C.; Banito, A.; Wuestefeld, T.; Georgilis, A.; Janich, P.; Morton, J.P.; Athineos, D.; Kang, T.-W.; Lasitschka, F.; Andrulis, M.; et al. A complex secretory program orchestrated by the inflammasome controls paracrine senescence. *Nat. Cell Biol.* **2013**, *15*, 978–990. [CrossRef] [PubMed]

45. Chen, H.; Ruiz, P.D.; McKimpson, W.M.; Novikov, L.; Kitsis, R.N.; Gamble, M.J. MacroH2A1 and ATM Play Opposing Roles in Paracrine Senescence and the Senescence-Associated Secretory Phenotype. *Mol. Cell* **2015**, *59*, 719–731. [CrossRef] [PubMed]
Hoare, M.; Ito, Y.; Kang, T.-W.; Weekes, M.P.; Matheson, N.J.; Patten, D.A.; Shetty, S.; Parry, A.J.; Menon, S.; Salama, R.; et al. NOTCH1 mediates a switch between two distinct secretomes during senescence. *Nat. Cell Biol.* **2016**, *18*, 979–992. [CrossRef] [PubMed]

Passos, J.F.; Nelson, G.; Wang, C.; Richter, T.; Simillion, C.; Proctor, C.J.; Miwa, S.; Olijslagers, S.; Hallinan, J.; Wipat, A.; et al. Feedback between p21 and reactive oxygen production is necessary for cell senescence. *Mol. Syst. Biol.* **2010**, *6*, 347. [CrossRef] [PubMed]

Hayakawa, T.; Iwai, M.; Aoki, S.; Takimoto, K.; Maruyama, M.; Maruyama, W.; Motoyama, N. SIRT1 suppresses the senescence-associated secretory phenotype through epigenetic gene regulation. *PLoS ONE* **2015**, *10*, 1–16. [CrossRef] [PubMed]

Lehmann, B.D.; Paine, M.S.; Brooks, A.M.; McCubrey, J.A.; Renegar, R.H.; Wang, R.; Terrian, D.M. Senescence-Associated Exosome Release from Human Prostate Cancer Cells. *Cancer Res.* **2008**, *68*, 7864–7871. [CrossRef] [PubMed]

Takasugi, M.; Okada, R.; Takahashi, A.; Virya Chen, D.; Watanabe, S.; Hara, E. Small extracellular vesicles secreted from senescent cells promote cancer cell proliferation through EphA8. *Nat. Commun.* **2017**, *8*, 15729. [CrossRef] [PubMed]

Webley, K.; Bond, J.A.; Jones, C.J.; Blaydes, J.P.; Craig, A.; Supinski, J.T.; Wynford-Thomas, D. Posttranslational modifications of p53 in replicative senescence overlapping but distinct from those induced by DNA damage. *Mol. Cell. Biol.* **2000**, *20*, 2803–2808. [CrossRef] [PubMed]

Tavana, O.; Benjamini, C.; Puebla-Osorio, N.; Sang, M.; Ullrich, S.E.; Ananthaswamy, H.N.; Zhu, C. Absence of p53-dependent apoptosis leads to UV radiation hypersensitivity, enhanced immunosuppression and cellular senescence. *Cell Cycle* **2010**, *9*, 3328–3336. [CrossRef] [PubMed]

Tang, Y.; Luo, J.; Zhang, W.; Gu, W. Tip60-Dependent Acetylation of p53 Modulates the Decision between Cell-Cycle Arrest and Apoptosis. *Mol. Cell* **2006**, *24*, 827–839. [CrossRef] [PubMed]

Lee, B.Y.; Han, J.A.; Im, J.S.; Morrone, A.; Johung, K.; Goodwin, E.C.; Kleijer, W.J.; DiMaio, D.; Hwang, E.S. Senescence-associated β-galactosidase is lysosomal β-galactosidase. *Aging Cell* **2006**, *5*, 187–195. [CrossRef] [PubMed]

Weichhart, T. mTOR as Regulator of Lifespan, Aging, and Cellular Senescence: A Mini-Review. *Gerontology* **2018**, *64*, 127–134. [CrossRef] [PubMed]

Höhn, A.; Grune, T. Lipofuscin: Formation, effects and role of macroautophagy. *Redox Biol.* **2013**, *1*, 140–144. [CrossRef] [PubMed]

Georgakopoulou, E.A.; Tsimaratou, K.; Evangelou, K.; Fernandez-Marcos, P.J.; Zoumpourlis, V.; Trougakos, I.P.; Kletsas, D.; Bartek, J.; Serrano, M.; Gorgoulis, V.G. Specific lipofuscin staining as a novel biomarker to detect replicative and stress-induced senescence. A method applicable in cryo-preserved and archival tissues. *Aging (Albany NY)* **2013**, *5*, 37–50. [CrossRef] [PubMed]

Evangelou, K.; Lougakiis, N.; Rizou, S.V.; Kotsinas, A.; Kletsas, D.; Muñoz-Espín, D.; Kastrinakis, N.G.; Pouli, N.; Marakis, P.; Townsend, P.; et al. Robust, universal biomarker assay to detect senescent cells in biological specimens. *Aging Cell* **2017**, *16*, 192–197. [CrossRef] [PubMed]

Wu, M.; Ye, H.; Shao, C.; Zheng, X.; Li, Q.; Wang, L.; Zhao, M.; Lu, G.; Chen, B.; Zhang, J.; et al. Metabolomics–Proteomics Combined Approach Identifies Differential Metabolism-Associated Molecular Events between Senescence and Apoptosis. *J. Proteome Res.* **2017**, *16*, 2250–2261. [CrossRef] [PubMed]

Correa-Melo, C.; Marques, F.D.; Anderson, R.; Hewitt, G.; Hewitt, R.; Cole, J.; Carroll, B.M.; Miwa, S.; Birch, J.; Merz, A.; et al. Mitochondria are required for pro-ageing features of the senescent phenotype. *EMBO J.* **2016**, *35*, 724–742. [CrossRef]

Lee, S.; Jeong, S.-Y.; Lim, W.-C.; Kim, S.; Park, Y.-Y.; Sun, X.; Youle, R.J.; Cho, H. Mitochondrial Fission and Fusion Mediators, hFis1 and OPA1, Modulate Cellular Senescence. *J. Biol. Chem.* **2007**, *282*, 22977–22983. [CrossRef] [PubMed]

Mai, S.; Klinkenberg, M.; Auburger, G.; Bereiter-Hahn, J.; Jendrach, M. Decreased expression of Drp1 and Fis1 mediates mitochondrial elongation in senescent cells and enhances resistance to oxidative stress through PINK1. *J. Cell Sci.* **2010**, *123*, 917–926. [CrossRef] [PubMed]

García-Prat, L.; Martínez-Vicente, M.; Perdigueró, E.; Ortet, L.; Rodríguez-Ubrea, J.; Rebollo, E.; Ruiz-Bonilla, V.; Gutarra, S.; Ballestar, E.; Serrano, A.L.; et al. Autophagy maintains stemness by preventing senescence. *Nature* **2016**, *529*, 37–42. [CrossRef] [PubMed]
64. Takahashi, Y.; Karbowski, M.; Yamaguchi, H.; Kazi, A.; Wu, J.; Sebti, S.M.; Youle, R.J.; Wang, H.-G. Loss of Bif-1 suppresses Bax/Bak conformational change and mitochondrial apoptosis. *Mol. Cell. Biol.* 2005, 25, 9369–9382. [CrossRef] [PubMed]

65. Correia-Melo, C.; Passos, J.F. Mitochondria: Are they causal players in cellular senescence? *Biochim. Biophys. Acta-Bioenerg.* 2015, 1847, 1373–1379. [CrossRef] [PubMed]

66. Passos, J.F.; Saretzki, G.; von Zglinicki, T. DNA damage in telomeres and mitochondria during cellular senescence: Is there a connection? *Nucleic Acids Res.* 2007, 35, 7505–7513. [CrossRef] [PubMed]

67. Tasselli, L.; Xi, Y.; Zheng, W.; Tennen, R.L.; Ondrowaz, Z.; Simeoni, F.; Li, W.; Chua, K.F. SIRT6 deacylates H3K18ac at pericentric chromatin to prevent mitotic errors and cellular senescence. *Nat. Struct. Mol. Biol.* 2016, 23, 434–440. [CrossRef] [PubMed]

68. Giblin, W.; Skinner, M.E.; Lombard, D.B. Sirtuins: Guardians of mammalian healthspan. *Trends Genet.* 2014, 30, 271–286. [CrossRef] [PubMed]

69. Komseli, E.-S.; Pateras, I.S.; Krejsgaard, T.; Stawiski, K.; Rizou, S.V.; Polyzos, A.; Roumelioti, F.-M.; Chourea, M.; Mourkioti, I.; Paparouna, E.; et al. A prototypical non-malignant epithelial model to study genome dynamics and concurrently monitor micro-RNAs and proteins in situ during oncogene-induced senescence. *BMC Genomics* 2018, 19, 37. [CrossRef] [PubMed]

70. Bannister, A.J.; Zegerman, P.; Partridge, J.F.; Miska, E.A.; Thomas, J.O.; Allshire, R.C.; Kouzarides, T. Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. *Nature* 2001, 410, 120–124. [CrossRef] [PubMed]

71. Dou, Z.; Ghosh, K.; Vizioli, M.G.; Zhu, J.; Sen, P.; Wangensteen, K.J.; Simithy, J.; Lan, Y.; Lin, Y.; Zhou, Z.; et al. Cytoplasmic chromatin triggers inflammation in senescence and cancer. *Nature* 2017, 550, 402–406. [CrossRef] [PubMed]

72. Di Micco, R.; Sulli, G.; Dobrev, M.; Lientos, M.; Botrugno, O.A.; Gargiulo, G.; dal Zuffo, R.; Matti, V.; d’Ario, G.; Montani, E.; et al. Interplay between oncogene-induced DNA damage response and heterochromatin in senescence and cancer. *Nat. Cell Biol.* 2011, 13, 292–302. [CrossRef] [PubMed]

73. Salama, R.; Sadaie, M.; Hoare, M.; Narita, M. Cellular senescence and its effector programs. *Genes Dev.* 2014, 28, 99–114. [CrossRef] [PubMed]

74. Freund, A.; Laberge, R.-M.; Demaria, M.; Campisi, J. Lamin B1 loss is a senescence-associated biomarker. *Mol. Biol. Cell* 2012, 23, 2066–2075. [CrossRef] [PubMed]

75. Cormenier, J.; Martin, N.; Desle, J.; Martin, N.; Saas, L.; Cormanier, J.; Malaquin, N.; Huot, L.; Slomianny, C.; Bouali, F.; et al. ATF6α regulates morphological changes associated with senescence in human fibroblasts. *Oncotarget* 2016, 7, 67699–67715. [PubMed]

76. Ohno-Iwashita, Y.; Shimada, Y.; Hayashi, M.; Inomata, M. Plasma membrane microdomains in aging and disease. *Geriatr. Gerontol. Int.* 2010, 10, S41–S52. [CrossRef] [PubMed]

77. Serrano, M.; Hannon, G.J.; Beach, D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. *Nature* 1993, 366, 704–707. [CrossRef] [PubMed]

78. Sharpless, N.E.; Sherr, C.J. Forging a signature of in vivo senescence. *Nat. Rev. Cancer* 2015, 15, 397–408. [CrossRef] [PubMed]

79. Zindy, F.; Quelle, D.E.; Roussel, M.F.; Sherr, C.J. Expression of the p16(INK4a) tumor suppressor versus other INK4 family members during mouse development and aging. *Oncogene* 1997, 15, 203–211. [CrossRef] [PubMed]

80. Burd, C.E.; Sorrentino, J.A.; Clark, K.S.; Darr, D.B.; Krishnamurthy, J.; Deal, A.M.; Bardeesy, N.; Castrillon, D.H.; Beach, D.H.; Sharpless, N.E. Monitoring tumorigenesis and senescence in vivo with a p16 INK4a-luciferase model. *Cell* 2013, 152, 340–351. [CrossRef] [PubMed]

81. Shapiro, G.; Edwards, C.; Kobzik, L. Reciprocal Rb Inactivation and p16 INK4 Expression in Primary Lung Cancers and Cell Lines. *Cancer Res.* 1995, 55, 505–509. [PubMed]
84. Witkiewicz, A.K.; Knudsen, K.E.; Dicker, A.P.; Knudsen, E.S. The meaning of p16 ink4a expression in tumors: Functional significance, clinical associations and future developments. *Cell Cycle* 2011, 10, 2497–2503. [CrossRef] [PubMed]
85. Sherr, C.J.; Roberts, J.M. CDK inhibitors: Positive and negative regulators of G1-phase progression. *Genes Dev.* 1999, 13, 1501–1512. [CrossRef] [PubMed]
86. Jung, Y.-S.; Qian, Y.; Chen, X. Examination of the expanding pathways for the regulation of p21 expression and activity. *Cell. Signal.* 2010, 22, 1003–1012. [CrossRef] [PubMed]
87. Dimri, G.P.; Lee, X.; Basile, G.; Acosta, M.; Scott, G.; Roskelley, C.; Medrano, E.E.; Linskens, M.; Rubelj, I.; Pereira-Smith, O. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc. Natl. Acad. Sci. USA* 1995, 92, 9363–9367. [CrossRef] [PubMed]
88. Kurz, D.J.; Decary, S.; Hong, Y.; Erusalimsky, J.D. Senescence-associated (beta)-galactosidase reflects an increase in lysosomal mass during replicative ageing of human endothelial cells. *J. Cell Sci.* 2000, 113, 3613–3622. [PubMed]
89. Severino, J.; Allen, R.G.; Balin, S.; Balin, A.; Cristofalo, V.J. Is beta-galactosidase staining a marker of senescence in vitro and in vivo? *Exp. Cell Res.* 2000, 257, 162–171. [CrossRef] [PubMed]
90. Gorgoulis, V.G.; Pefani, D.; Pateras, I.S.; Trougakos, I.P. Integrating the DNA damage and protein stress responses during cancer development and treatment. *J. Pathol.* 2018. [CrossRef] [PubMed]
91. Terman, A.; Brunk, U.T. Lipofuscin. *Lipofuscin: Formation, distribution, and metabolic consequences.* 2018, in press.
92. Nelson, G.; Wordsworth, J.; Wang, C.; Jurk, D.; Lawless, C.; Martin-Ruiz, C.; von Zglinicki, T. A senescent cell bystander effect: Senescence-induced senescence. *Aging Cell* 2012, 11, 345–349. [CrossRef] [PubMed]
93. Ribezzo, F.; Shiloh, Y.; Schumacher, B. Systemic DNA damage responses in aging and diseases. *Semin. Cancer Biol.* 2016, 37–38, 26–35. [CrossRef] [PubMed]
94. Bauer, J.; Strauss, S.; Schreiter-Gasser, U.; Ganter, U.; Schlegel, P.; Witt, I.; Yolk, B.; Berger, M. Interleukin-6 and alpha-2-macroglobulin indicate an acute-phase state in Alzheimer’s disease cortices. *FEBS Lett.* 1991, 285, 111–114. [CrossRef]
95. Huell, M.; Strauss, S.; Volk, B.; Berger, M.; Bauer, J. Interleukin-6 is present in early stages of plaque formation and is restricted to the brains of Alzheimer’s disease patients. *Acta Neuropathol.* 1995, 89, 544–551. [CrossRef] [PubMed]
96. Kiecolt-Glaser, J.K.; Preacher, K.J.; MacCallum, R.C.; Atkinson, C.; Malarkey, W.B.; Glaser, R. Chronic stress and age-related increases in the proinflammatory cytokine IL-6. *Proc. Natl. Acad. Sci. USA* 2003, 100, 9090–9095. [CrossRef] [PubMed]
97. Campbell, I.L.; Abraham, C.R.; Masliah, E.; Kemper, P.; Inglis, J.D.; Oldstone, M.B.; Mucke, L. Neurologic disease induced in transgenic mice by cerebral overexpression of interleukin 6. *Proc. Natl. Acad. Sci. USA* 1993, 90, 10061–10065. [CrossRef] [PubMed]
98. Streit, W.J.; Sammons, N.W.; Kuhns, A.J.; Sparks, D.L. Dystrophic Microglia in the Aging Human Brain. *Glia* 2004, 45, 208–212. [CrossRef] [PubMed]
99. Rawji, K.S.; Mishra, M.K.; Michaels, N.J.; Rivest, S.; Stys, P.K.; Yong, V.W. Immunosenescence of microglia and macrophages: Impact on the ageing central nervous system. *Brain* 2016, 139, 653–661. [CrossRef] [PubMed]
100. Kiecolt-Glaser, J.K.; Preacher, K.J.; MacCallum, R.C.; Atkinson, C.; Malarkey, W.B.; Glaser, R. Chronic stress and age-related increases in the proinflammatory cytokine IL-6. *Proc. Natl. Acad. Sci. USA* 2003, 100, 9090–9095. [CrossRef] [PubMed]
101. Kiecolt-Glaser, J.K.; Preacher, K.J.; MacCallum, R.C.; Atkinson, C.; Malarkey, W.B.; Glaser, R. Chronic stress and age-related increases in the proinflammatory cytokine IL-6. *Proc. Natl. Acad. Sci. USA* 2003, 100, 9090–9095. [CrossRef] [PubMed]
106. Calabrese, V.; Santoro, A.; Monti, D.; Crupi, R.; Di Paola, R.; Latteri, S.; Cuzzocrea, S.; Zappia, M.; Giordano, J.; Calabrese, E.J.; et al. Aging and Parkinson’s Disease: Inflammaging, neuroinflammation and biological remodeling as key factors in pathogenesis. *Free Radic. Biol. Med.* 2018, 115, 80–91. [CrossRef] [PubMed]

107. Ming, G.; Song, H. Adult Neurogenesis in the Mammalian Brain: Significant Answers and Significant Questions. *Neuron* 2011, 70, 687–702. [CrossRef] [PubMed]

108. Kriegstein, A.; Alvarez-Buylla, A. The Glial Nature of Embryonic and Adult Neural Stem Cells. *Annu. Rev. Neurosci.* 2009, 32, 149–184. [CrossRef] [PubMed]

109. Cipriani, S.; Ferrer, I.; Aronica, E.; Kovacs, G.G.; Verney, C.; Nardelli, J.; Khung, S.; Delezoide, A.L.; Milenkovic, I.; Rasika, S.; et al. Hippocampal Radial Glial Subtypes and Their Neurogenic Potential in Human Fetuses and Healthy and Alzheimer’s Disease Adults. *Cereb Cortex* 2018, 28, 2458–2478. [CrossRef] [PubMed]

110. Yang, Z.; Jun, H.; Choi, C.-I.; Yoo, K.H.; Cho, C.H.; Hussaini, S.M.Q.; Simmons, A.J.; Kim, S.; van Deursen, J.M.; Baker, D.J.; et al. Age-related decline in BubR1 impairs adult hippocampal neurogenesis. *Aging Cell* 2017, 16, 598–601. [CrossRef] [PubMed]

111. Hollands, C.; Tobin, M.K.; Hsu, M.; Musaraca, K.; Yu, T.-S.; Mishra, R.; Kernie, S.G.; Lazarov, O. Depletion of adult neurogenesis exacerbates cognitive deficits in Alzheimer’s disease by compromising hippocampal inhibition. *Mol. Neurodegener.* 2017, 12, 64. [CrossRef] [PubMed]

112. Lee, I.-S.; Jung, K.; Kim, I.-S.; Lee, H.; Kim, M.; Yun, S.; Hwang, K.; Shin, J.E.; Park, K.I. Human neural stem cells alleviate Alzheimer-like pathology in a mouse model. *Mol. Neurodegener.* 2015, 10, 38. [CrossRef] [PubMed]

113. Reynolds, R.; Dawson, M.; Papadopoulos, D.; Polito, A.; Di Bello, I.C.; Pham-Dinh, D.; Levine, J. The response of NG2-expressing oligodendrocyte progenitors to demyelination in MOG-EAE and MS. *J. Neurocytol.* 2002, 31, 523–536. [CrossRef] [PubMed]

114. Chang, A.; Tourtellotte, W.W.; Rudick, R.; Trapp, B.D. Premyelinating Oligodendrocytes in Chronic Lesions of Multiple Sclerosis. *N. Engl. J. Med.* 2002, 346, 165–173. [CrossRef] [PubMed]

115. Prineas, J.W.; Barnard, R.O.; Kwon, E.E.; Sharer, L.R.; Cho, E.-S. Multiple sclerosis: Remyelination of nascent lesions. *Neurology* 1993, 43, 137–151. [CrossRef] [PubMed]

116. Sim, F.J.; Zhao, C.; Penderis, J.; Franklin, R.J.M. The age-related decrease in CNS remyelination efficiency is attributable to an impairment of both oligodendrocyte progenitor recruitment and differentiation. *J. Neurosci.* 2002, 22, 2451–2459. [CrossRef] [PubMed]

117. Kornek, B.; Storch, M.K.; Weissert, R.; Wallstroem, E.; Stefferl, A.; Olsson, T.; Linnington, C.; Schmidbauer, M.; Lassmann, H. Multiple Sclerosis and Chronic Autoimmune Encephalomyelitis. *Am. J. Pathol.* 2000, 157, 267–276. [CrossRef]

118. Patrikios, P.; Stadelmann, C.; Kutzelnigg, A.; Rauschka, H.; Schmidbauer, M.; Laursen, H.; Sorensen, P.S.; Bruck, W.; Luchinetti, C.; Lassmann, H. Remyelination is extensive in a subset of multiple sclerosis patients. *Brain* 2006, 129, 3165–3172. [CrossRef] [PubMed]

119. Purcell, M.; Kruger, A.; Tainsky, M.A. Gene expression profiling of replicative and induced senescence. *Cell Cycle* 2014, 13, 3927–3937. [CrossRef] [PubMed]

120. De Stefano, N.; Stromillo, M.L.; Giorgio, A.; Bartolozzi, M.L.; Battaglini, M.; Baldini, M.; Portaccio, E.; Amato, M.P.; Sormani, M.P. Establishing pathological cut-offs of brain atrophy rates in multiple sclerosis. *J. Neurol. Neurosurg. Psychiatry* 2009, 87, 93–99. [CrossRef] [PubMed]

121. Hawkins, B.T.; Davis, T.P. The blood-brain barrier/neurovascular unit in health and disease. *Pharmacol. Rev.* 2005, 57, 173–185. [CrossRef] [PubMed]

122. Brown, W.R.; Thore, C.R. Review: Cerebral microvascular pathology in ageing and neurodegeneration. *Neuropathol. Appl. Neurobiol.* 2011, 37, 56–74. [CrossRef] [PubMed]

123. Bell, R.D.; Zlokovic, B.V. Neurovascular mechanisms and blood-brain barrier disorder in Alzheimer’s disease. *Acta Neuropathol.* 2009, 118, 103–113. [CrossRef] [PubMed]

124. Farrall, A.J.; Wardlaw, J.M. Blood-brain barrier: Ageing and microvascular disease—systematic review and meta-analysis. *Neurobiol. Aging* 2009, 30, 337–352. [CrossRef] [PubMed]

125. van de Haar, H.J.; Burgmans, S.; Jansen, J.F.; van Ooij, M.J.; van Buchem, M.A.; Muller, M.; Hofman, P.A.; Verhey, F.R.; Backes, W.H. Blood-Brain Barrier Leakage in Patients with Early Alzheimer Disease. *Radiology* 2016, 281, 527–535. [CrossRef] [PubMed]
126. Ujie, M.; Dickstein, D.L.; Carlow, D.A.; Jefferies, W.A. Blood-brain barrier permeability precedes senile plaque formation in an Alzheimer disease model. Microcirculation 2003, 10, 463–470. [PubMed]

127. Zipser, B.D.; Johanson, C.E.; Gonzalez, L.; Berzin, T.M.; Tavares, R.; Hulette, C.M.; Vitek, M.P.; Hovanesian, V.; Stopa, E.G. Microvascular injury and blood-brain barrier leakage in Alzheimer’s disease. Neurobiol. Aging 2007, 28, 977–986. [CrossRef] [PubMed]

128. Erusalimskey, J.D. Vascular endothelial senescence: From mechanisms to pathophysiology. J. Appl. Physiol. 2009, 106, 326–332. [CrossRef] [PubMed]

129. Yamazaki, Y.; Baker, D.J.; Tachibana, M.; Liu, C.C.; van Deursen, J.M.; Brott, T.G.; Bu, G.; Kanekiyo, T. Vascular Cell Senescence Contributes to Blood-Brain Barrier Breakdown. Stroke 2016, 47, 1068–1077. [CrossRef] [PubMed]

130. Stamatanovic, S.; Martinez, G.; Hu, A.; Choi, J.; Keep, R.; Andjelkovic, A. Decline in Sirtuin-1 expression and activity plays a critical role in blood-brain barrier permeability in aging. Neurobiol. Dis. 2018, pii: S0969-9961(18)30555-2. [CrossRef]

131. Rubenstein, E. Relationship of senescence of cerebrospinal fluid circulatory system to dementias of the aged. Lancet 1998, 351, 283–285. [CrossRef]

132. Ott, B.R.; Jones, R.N.; Daiello, L.A.; de la Monte, S.M.; Stopa, E.G.; Dey, C.; Grammas, P. Blood-Cerebrospinal Fluid Barrier Gradients in Mild Cognitive Impairment and Alzheimer’s Disease: Relationship to Inflammatory Cytokines and Chemokines. Front Aging Neurosci. 2018, 10, 245. [CrossRef] [PubMed]

133. Kivisäkk, P.; Mahad, D.J.; Callahan, M.K.; Trebst, C.; Tucky, B.; Wei, T.; Wu, L.; Baekkevold, E.S.; Lassmann, H.; Staugaitis, S.M.; et al. Human cerebrospinal fluid central memory CD4+ T cells: Evidence for trafficking through choroid plexus and meninges via P-selectin. Proc. Natl. Acad. Sci. USA 2003, 100, 8389–8394. [CrossRef] [PubMed]

134. Praetorius, J.; Damkier, H.H. Transport across the choroid plexus epithelium. Am. J. Physiol. Cell Physiol. 2017, 312, C673–C686. [CrossRef] [PubMed]

135. Baruch, K.; Deczkowska, A.; David, E.; Castellano, J.M.; Miller, O.; Kertser, A.; Berkutzki, T.; Barnett-Itzhaki, Z.; Bezalel, D.; Wyss-Coray, T.; et al. Aging. Aging-induced type I interferon response at the choroid plexus negatively affects brain function. Science 2014, 346, 89–93. [CrossRef] [PubMed]

136. Sofroniew, M.V.; Vinters, H.V. Astrocytes: Biology and pathology. Acta Neuropathol. 2010, 119, 7–35. [CrossRef] [PubMed]

137. Mansour, H.; Chamberlain, C.G.; Weible, M.W.; Hughes, S.; Chu, Y.; Chan-Ling, T. Aging-related changes in astrocytes in the rat retina: Imbalance between cell proliferation and cell death reduces astrocyte availability. Aging Cell 2008, 7, 526–540. [CrossRef] [PubMed]

138. Rubenstein, E. Relationship of senescence of cerebrospinal fluid circulatory system to dementias of the aged. Lancet 1998, 351, 283–285. [CrossRef]

139. Yamazaki, Y.; Baker, D.J.; Tachibana, M.; Liu, C.C.; van Deursen, J.M.; Brott, T.G.; Bu, G.; Kanekiyo, T. Vascular Cell Senescence Contributes to Blood-Brain Barrier Breakdown. Stroke 2016, 47, 1068–1077. [CrossRef] [PubMed]

140. Lee, J.-H.; Yu, W.H.; Lee, S.; Mohan, P.S.; Peterhoff, C.M.; Wolfe, D.M.; Martinez-Vicente, M.; Massey, A.C.; Sovak, G.; et al. Lysosomal Proteolysis and Autophagy Require Presenilin 1 and Are Disrupted through Alzheimer-Related PS1 Mutations. Cell 2010, 141, 1146–1158. [CrossRef] [PubMed]

141. Evans, R.J.; Wyllie, F.S.; Wyllie, F.S.; Wynford-Thomas, D.; Kipling, D.; Jones, C.J. A PS3-dependent, telomere-independent proliferative life span barrier in human astrocytes consistent with the molecular genetics of glia A. Cell 2007, 101, 794–805. [CrossRef] [PubMed]

142. Lee, J.-H.; Yu, W.H.; Kumar, A.; Lee, S.; Mohan, P.S.; Peterhoff, C.M.; Wolfe, D.M.; Martinez-Vicente, M.; Massey, A.C.; Sovak, G.; et al. Lysosomal Proteolysis and Autophagy Require Presenilin 1 and Are Disrupted through Alzheimer-Related PS1 Mutations. Cell 2010, 141, 1146–1158. [CrossRef] [PubMed]

143. Nie, X.; Liang, L.; Xi, H.; Jiang, S.; Jiang, J.; Tang, C.; Liu, X.; Liu, S.; Wan, C.; Zhao, J.; et al. 2,3,7,8-Tetrachlorodibenzo-p-dioxin induces premature senescence of astrocytes via WNT/β-catenin signaling and ROS production. J. Appl. Toxicol. 2015, 35, 851–860. [CrossRef] [PubMed]

144. Görg, B.; Karababa, A.; Shafigullina, A.; Bidmon, H.J.; Häussinger, D. Ammonia-induced senescence in cultured rat astrocytes and in human cerebral cortex in hepatic encephalopathy. Glia 2015, 63, 37–50. [CrossRef] [PubMed]
145. Chinta, S.J.; Woods, G.; Demaria, M.; Rane, A.; Zou, Y.; Mcquade, A.; Rajagopalan, S.; Limbad, C.; Madden, D.T.; Campisi, J.; et al. Cellular Senescence Is Induced by the Environmental Neurotoxin Paraquat and Contributes to Neuropathology Linked to Parkinson’s Disease. *Cell Rep.* 2018, 22, 930–940. [CrossRef] [PubMed]

146. Goldman, S.M. Environmental Toxins and Parkinson’s Disease. *Annu. Rev. Pharmacol. Toxicol.* 2014, 54, 141–164. [CrossRef] [PubMed]

147. Crowe, E.P.; Tuzer, F.; Gregory, B.D.; Donahue, G.; Gosai, S.J.; Cohen, J.; Leung, Y.Y.; Yetkin, E.; Nativio, R.; Wang, L.-S.; et al. Changes in the Transcriptome of Human Astrocytes Accompanying Oxidative Stress-Induced Senescence. *Front. Aging Neurosci.* 2016, 8, 208. [CrossRef] [PubMed]

148. Bhat, R.; Crowe, E.P.; Bitto, A.; Moh, M.; Katsetos, C.D.; Garcia, F.U.; Johnson, F.B.; Trojanowski, J.Q.; Sell, C.; Torres, C. Astrocyte Senescence as a Component of Alzheimer’s Disease. *PLoS ONE* 2012, 7, 1–10. [CrossRef] [PubMed]

149. Hou, J.; Cui, C.; Kim, S.; Sung, C.; Choi, C. Ginsenoside F1 suppresses astrocytic senescence-associated secretory phenotype. *Chem. Biol. Interact.* 2018, 283, 75–83. [CrossRef] [PubMed]

150. Mombach, J.C.M.M.; Vendrusculo, B.; Bugs, C.A. A model for p38MAPK-induced astrocyte senescence. *PLoS ONE* 2015, 10, 1–12. [CrossRef] [PubMed]

151. Hartman, T.K.; Wengenack, T.M.; Poduslo, J.F.; van Deursen, J.M. Mutant mice with small amounts of BubR1 display accelerated age-related gliosis. *Neurobiol. Aging* 2007, 28, 921–927. [CrossRef] [PubMed]

152. Ransohoff, R.M.; Brown, M.A. Innate immunity in the central nervous system. *J. Clin. Invest.* 2012, 122, 1164–1171. [CrossRef] [PubMed]

153. Flanary, B.E.; Streit, W.J. Progressive Telomere Shortening Occurs in Cultured Rat Microglia, but Not Astrocytes. *Glia* 2004, 45, 75–88. [CrossRef] [PubMed]

154. Flanary, B.E.; Sammons, N.W.; Nguyen, C.; Walker, D.; Streit, W.J. Evidence That Aging and Amyloid Promote Microglial Cell Senescence. *Rejuvenation Res.* 2007, 10, 61–74. [CrossRef] [PubMed]

155. Yu, H.-M.; Zhao, Y.-M.; Luo, X.-G.; Feng, Y.; Ren, Y.; Shang, H.; He, Z.-Y.; Luo, X.-M.; Chen, S.-D.; Wang, X.-Y. Repeated Lipopolysaccharide Stimulation Induces Cellular Senescence in BV2 Cells. *Neuroimmunomodulation* 2012, 19, 131–136. [CrossRef] [PubMed]

156. Conde, J.R.; Streit, W.J. Microglia in the Aging Brain. *J. Neuropathol. Exp. Neurol.* 2006, 65, 199–203. [CrossRef] [PubMed]

157. Streit, W.J.; Braak, H.; Xue, Q.-S.; Bechmann, I.; Qing-Shan, A.; Ae, X.; Bechmann, I. Dystrophic (senescent) rather than activated microglial cells are associated with tau pathology and likely precede neurodegeneration in Alzheimer’s disease. *Acta Neuropathol.* 2009, 118, 475–485. [CrossRef] [PubMed]

158. Clarke, L.E.; Liddelow, S.A.; Chakraborty, C.; Münch, A.E.; Heiman, M.; Barres, B.A. Normal aging induces A1-like astrocyte reactivity. *Proc. Natl. Acad. Sci. USA* 2018, 115, E1896–E1905. [CrossRef] [PubMed]

159. Giacci, M.K.; Bartlett, C.A.; Smith, N.M.; Iyer, K.S.; Toomey, L.M.; Jiang, H.; Guagliardo, P.; Kilburn, M.R.; Fitzgerald, M. Oligodendroglia Are Particularly Vulnerable to Oxidative Damage after Neurotrauma In Vivo. *J. Neurosci.* 2018, 38, 6491–6504. [CrossRef] [PubMed]

160. Al-Mashhadi, S.; Simpson, J.E.; Heath, P.R.; Dickman, M.; Forster, G.; Matthews, F.E.; Brayne, C.; Ince, P.G.; Wharton, S.B. Oxidative glial cell damage associated with white matter lesions in the aging human brain. *Brain Pathol.* 2015, 25, 565–574. [CrossRef] [PubMed]

161. Brickman, A.M. Contemplating Alzheimer’s Disease and the Contribution of White Matter Hyperintensities. *Curr. Neurol. Neurosci. Rep.* 2013, 13, 415. [CrossRef] [PubMed]

162. Gouw, A.A.; van der Flier, W.M.; Fazekas, F.; van Straaten, E.C.W.; Pantoni, L.; Poggesi, A.; Inzitari, D.; Erkinjuntti, T.; Wahlund, L.O.; Waldemar, G.; et al. Progression of White Matter Hyperintensities and Incidence of New Lacunes Over a 3-Year Period: The Leukoaraiosis and Disability Study. *Stroke* 2008, 39, 1414–1420. [CrossRef] [PubMed]

163. Erten-Lyons, D.; Woltjer, R.; Kaye, J.; Mattek, N.; Dodge, H.H.; Green, S.; Tran, H.; Howieson, D.B.; Wild, K.; Silbert, L.C. Neuropathologic basis of white matter hyperintensity accumulation with advanced age. *Neurology* 2013, 81, 977–983. [CrossRef] [PubMed]

164. Nasrabady, S.E.; Rizvi, B.; Goldman, J.E.; Brickman, A.M. White matter changes in Alzheimer’s disease: A focus on myelin and oligodendrocytes. *Acta Neuropathol. Commun.* 2018, 6, 22. [CrossRef] [PubMed]
165. Dawson, M.R.L.; Polito, A.; Levine, J.M.; Reynolds, R. NG2-expressing glial progenitor cells: An abundant and widespread population of cycling cells in the adult rat CNS. Mol. Cell. Neurosci. 2003, 24, 476–488. [CrossRef]

166. Tang, D.G.; Tokumoto, Y.M.; Apperly, J.A.; Lloyd, A.C.; Raff, M.C. Lack of replicative senescence in cultured rat oligodendrocyte precursor cells. Science (80-.) 2001, 291, 868–871. [CrossRef] [PubMed]

167. Kijuro, Y.; Suzuki, N.; Kondo, T. Esophageal cancer-related gene 4 is a secreted inducer of cell senescence expressed by aged CNS precursor cells. Proc. Natl. Acad. Sci. U.S.A. 2010, 107, 8259–8264. [CrossRef] [PubMed]

168. Luccinetti, C.; Brück, W.; Parisi, J.; Scheithauer, B.; Rodríguez, M.; Lassmann, H. A quantitative analysis of oligodendrocytes in multiple sclerosis lesions. A study of 113 cases. Brain 1999, 122, 2279–2295. [CrossRef] [PubMed]

169. Patani, R.; Balaratnam, M.; Vora, A.; Reynolds, R. Remyelination can be extensive in multiple sclerosis despite a long disease course. Neuropathol. Appl. Neurobiol. 2007, 33, 277–287. [CrossRef] [PubMed]

170. Choi, C.-I.; Yoo, K.H.; Hussaini, S.M.Q.; Jeon, B.T.; Welby, J.; Gan, H.; Scarisbrick, I.A.; Zhang, Z.; Baker, D.J.; He, N.; Jin, W.-L.; Lok, K.-H.; Wang, Y.; Yin, M.; Wang, Z.-J. Amyloid-β oligomer accelerates senescence in adult hippocampal neural stem/progenitor cells via formylpeptide receptor 2. Cell Death Dis. 2013, 4, e924. [CrossRef] [PubMed]

171. Li, W.-Q.; Wang, Z.; Liu, S.; Hu, Y.; Yin, M.; Lu, Y. N-Stearoyl-L-Tyrosine Inhibits the Senescence of Neural Stem/Progenitor Cells Induced by Aβ 1-42 via the CB2 Receptor. Stem Cells Int. 2016, 2016, 1–9.

172. Bose, R.; Moors, M.; Tofgighi, R.; Cascante, A.; Hermanson, O.; Ceccatelli, S. Glucocorticoids induce long-lasting effects in neural stem cells resulting in senescence-related alterations. Cell Death Dis. 2010, 1, e92. [CrossRef] [PubMed]

173. Ferron, S.R.; Marques-Torrejon, M.A.; Mira, H.; Flores, I.; Taylor, K.; Blasco, M.A.; Farinas, I. Telomere Shortening in Neural Stem Cells Disrupts Neuronal Differentiation and Neuritogenesis. J. Neurosci. 2009, 29, 14394–14407. [CrossRef] [PubMed]

174. Selkoe, D.J.; Hardy, J. The amyloid hypothesis of Alzheimer’s disease at 25 years. EMBO Mol. Med. 2016, 8, 595–608. [CrossRef] [PubMed]

175. Magini, A.; Polchi, A.; Tozzi, A.; Tancini, B.; Tantucci, M.; Urbaneli, L.; Bonsello, T.; Calabresi, P.; Emiliani, C. Abnormal cortical lysosomal β-hexosaminidase and β-galactosidase activity at post-synaptic sites during Alzheimer’s disease progression. Int. J. Biochem. Cell Biol. 2015, 58, 62–70. [CrossRef] [PubMed]
185. Tribuzi, R.; Orlacchio, A.A.; Crispoltoni, L.; Maiotti, M.; Zampolini, M.; De Angelis, M.; Mecocci, P.; Cecchetti, R.; Bernardi, G.; Datti, A.; et al. Lysosomal β-galactosidase and β-hexosaminidase activities correlate with clinical stages of dementia associated with Alzheimer’s disease and type 2 diabetes mellitus. J. Alzheimer’s Dis. 2011, 24, 785–797. [CrossRef] [PubMed]

186. Tribuzi, R.; Crispoltoni, L.; Porcellati, S.; Di Lullo, M.; Florenzano, F.; Pirro, M.; Bagaglia, F.; Kawarai, T.; Zampolini, M.; Orlacchio, A.A.; et al. MiR128 up-regulation correlates with impaired amyloid β(1–42) degradation in monocytes from patients with sporadic Alzheimer’s disease. Neurobiol. Aging 2014, 35, 345–356. [CrossRef] [PubMed]

187. Vincent, I.; Rosado, M.; Davies, P. Mitotic mechanisms in Alzheimer’s disease? J. Cell Biol. 1996, 132, 413–425. [CrossRef] [PubMed]

188. Herrup, K.; Yang, Y. Cell cycle regulation in the postmitotic neuron: Oxymoron or new biology? Nat. Rev. Neurosci. 2007, 8, 368–378. [CrossRef] [PubMed]

189. Nagy, Z. The last neuronal division: A unifying hypothesis for the pathogenesis of Alzheimer’s disease. J. Cell. Mol. Med. 2005, 9, 531–541. [CrossRef] [PubMed]

190. Currais, A.; Hortobágyi, T.; Soriano, S. The neuronal cell cycle as a mechanism of pathogenesis in Alzheimer’s. Aging (Albany NY) 2009, 1, 363–371. [CrossRef] [PubMed]

191. Chang, B.D.; Watanabe, K.; Broude, E.V.; Fang, J.; Poole, J.C.; Kalinichenko, T.V.; Roninson, I.B. Effects of regulators P16 and CDK4 in Alzheimer’s disease. Proc. Natl. Acad. Sci. USA 2000, 97, 4291–4296. [CrossRef] [PubMed]

192. Engidawork, E.; Gulesserian, T.; Seidl, R.; Cairns, N.; Lubec, G. Expression of apoptosis related proteins in brains of patients with Alzheimer’s disease. Neurosci. Lett. 2001, 303, 79–82. [CrossRef] [PubMed]

193. Currais, A.; Hortobágyi, T.; Soriano, S. The neuronal cell cycle as a mechanism of pathogenesis in Alzheimer’s. Aging (Albany NY) 2009, 1, 363–371. [CrossRef] [PubMed]

194. Esteras, N.; Bartolomé, F.; Alquézar, C.; Antequera, D.; Muñoz, Ú.; Carro, E.; Martín-Requero, Á. Altered cell cycle-related gene expression in brain and lymphocytes from a transgenic mouse model of Alzheimer’s disease [amyloid precursor protein/presenilin 1 (PS1)]. Eur. J. Neurosci. 2012, 36, 2609–2618. [CrossRef] [PubMed]

195. Tiribuzi, R.; Orlacchio, A.A.; Crispoltoni, L.; Maiotti, M.; Zampolini, M.; De Angelis, M.; Mecocci, P.; Cecchetti, R.; Bernardi, G.; Datti, A.; et al. Lysosomal β-galactosidase and β-hexosaminidase activities correlate with clinical stages of dementia associated with Alzheimer’s disease and type 2 diabetes mellitus. J. Alzheimer’s Dis. 2011, 24, 785–797. [CrossRef] [PubMed]

196. Delobel, P.; Lavenir, I.; Ghetti, B.; Holzer, M.; Goedert, M. Cell-Cycle Markers in a Transgenic Mouse Model of Human Tauopathy. J. Neural Transm. 1998, 105, 949–960. [CrossRef] [PubMed]

197. Esteras, N.; Bartolomé, F.; Alquézar, C.; Antequera, D.; Muñoz, Ú.; Carro, E.; Martín-Requero, Á. Altered cell cycle-related gene expression in brain and lymphocytes from a transgenic mouse model of Alzheimer’s disease [amyloid precursor protein/presenilin 1 (PS1)]. Eur. J. Neurosci. 2012, 36, 2609–2618. [CrossRef] [PubMed]

198. Delobel, P.; Lavenir, I.; Ghetti, B.; Holzer, M.; Goedert, M. Cell-Cycle Markers in a Transgenic Mouse Model of Human Tauopathy. Am. J. Pathol. 2006, 168, 878–887. [CrossRef] [PubMed]

199. Tan, M.; Wang, S.; Song, J.; Jia, J. Combination of p53(ser15) and p21/p21(thr145) in peripheral blood lymphocytes as potential Alzheimer’s disease biomarkers. Neurosci. Lett. 2012, 516, 226–231. [CrossRef] [PubMed]

200. Tiribuzi, R.; Orlacchio, A.A.; Crispoltoni, L.; Maiotti, M.; Zampolini, M.; De Angelis, M.; Mecocci, P.; Cecchetti, R.; Bernardi, G.; Datti, A.; et al. Lysosomal β-galactosidase and β-hexosaminidase activities correlate with clinical stages of dementia associated with Alzheimer’s disease and type 2 diabetes mellitus. J. Alzheimer’s Dis. 2011, 24, 785–797. [CrossRef] [PubMed]

201. Arendt, T.; Rödel, L.; Gärtner, U.; Holzer, M. Expression of the cyclin-dependent kinase inhibitor p16 in Alzheimer’s disease. Neuroreport 1999, 7, 3047–3049. [PubMed]

202. Tiribuzi, R.; Orlacchio, A.A.; Crispoltoni, L.; Maiotti, M.; Zampolini, M.; De Angelis, M.; Mecocci, P.; Cecchetti, R.; Bernardi, G.; Datti, A.; et al. Lysosomal β-galactosidase and β-hexosaminidase activities correlate with clinical stages of dementia associated with Alzheimer’s disease and type 2 diabetes mellitus. J. Alzheimer’s Dis. 2011, 24, 785–797. [CrossRef] [PubMed]

203. Arendt, T.; Rödel, L.; Gärtner, U.; Holzer, M. Aberrant expression of the cyclin-dependent kinase inhibitor p16 in Alzheimer’s disease. Neuroreport 1999, 7, 3047–3049. [PubMed]

204. Tiribuzi, R.; Orlacchio, A.A.; Crispoltoni, L.; Maiotti, M.; Zampolini, M.; De Angelis, M.; Mecocci, P.; Cecchetti, R.; Bernardi, G.; Datti, A.; et al. Lysosomal β-galactosidase and β-hexosaminidase activities correlate with clinical stages of dementia associated with Alzheimer’s disease and type 2 diabetes mellitus. J. Alzheimer’s Dis. 2011, 24, 785–797. [CrossRef] [PubMed]

205. Arendt, T.; Rödel, L.; Gärtner, U.; Holzer, M. Aberrant expression of the cell cycle regulators P16 and CDK4 in Alzheimer’s disease. Am. J. Pathol. 1997, 150, 1933–1939. [PubMed]
223. Leake, A.; Morris, C.; Whateley, J. Brain matrix metalloproteinase 1 levels are elevated in Alzheimer’s disease. *Neurosci. Lett.* **2000**, 291, 201–203. [CrossRef] [PubMed]

224. Bjerke, M.; Zetterberg, H.; Edman, Å.; Blennow, K.; Wallin, A.; Andreasson, U. Cerebrospinal fluid matrix metalloproteinases and tissue inhibitor of metalloproteinases in combination with subcortical and cortical biomarkers in vascular dementia and Alzheimer’s disease. *J. Alzheimer’s Dis.* **2011**, 27, 665–676. [CrossRef] [PubMed]

225. Horstmann, S.; Budig, L.; Gardner, H.; Koziol, J.; Deuschle, M.; Schilling, C.; Wagner, S. Matrix metalloproteinases in peripheral blood and cerebrospinal fluid in patients with Alzheimer’s disease. *Int. Psychogeriatr.* **2018**, 226, 966–972. [CrossRef] [PubMed]

226. Yoshiyama, Y.; Asahina, M.; Hattori, T. Selective distribution of matrix metalloproteinase-3 (MMP-3) in Alzheimer’s disease brain. *Acta Neuropathol.* **2000**, 99, 91–95. [CrossRef] [PubMed]

227. Qazi, T.J.; Quan, Z.; Mir, A.; Qing, H. Epigenetics in Alzheimer’s Disease: Perspective of DNA Methylation. *Mol. Neurobiol.* **2018**, 55, 1026–1044. [CrossRef] [PubMed]

228. Watson, C.T.; Roussos, P.; Garg, P.; Ho, D.J.; Azam, N.; Katsel, P.L.; Haroutunian, V.; Sharp, A.J. Genome-wide DNA methylation profiling in the superior temporal gyrus reveals epigenetic signatures associated with Alzheimer’s disease. *Genome Med.* **2016**, 8, 5. [CrossRef] [PubMed]

229. Lord, J.; Cruchaga, C. The epigenetic landscape of Alzheimer’s disease. *Nat. Neurosci.* **2014**, 17, 1138–1140. [CrossRef] [PubMed]

230. Silva, A.R.T.; Santos, A.C.F.; Farfel, J.M.; Grinberg, L.T.; Ferretti, R.E.L.; Campos, A.H.J.F.M.; Cunha, I.W.; Begnami, M.D.; Rocha, R.M.; Carraro, D.M.; et al. Repair of Oxidative DNA Damage, Cell-Cycle Regulation and Neuronal Death May Influence the Clinical Manifestation of Alzheimer’s Disease. *PLoS ONE* **2014**, 9, e99897. [CrossRef] [PubMed]

231. Siddiqui, M.S.; Francois, M.; Hecker, J.; Faunt, J.; Fenech, M.F.; Leifert, W.R. γH2AX is increased in peripheral blood lymphocytes of Alzheimer’s disease patients in the South Australian Neurodegeneration, Nutrition and DNA Damage (SAND) study of aging. *Mutat. Res.-Genet. Toxicol. Environ. Mutagen.* **2018**, 829–830, 6–18. [CrossRef] [PubMed]

232. Nixon, R.A. The role of autophagy in neurodegenerative disease. *Nat. Med.* **2013**, 19, 983–997. [CrossRef] [PubMed]

233. Chung, K.M.; Hernández, N.; Sproul, A.; Yu, W.H. Alzheimer’s disease and the autophagic-lysosomal system. *Neurosci. Lett.* **2018**. [CrossRef] [PubMed]

234. Ihara, Y.; Morishima-Kawashima, M.; Nixon, R. The ubiquitin-proteasome system and the autophagic-lysosomal system in Alzheimer disease. *Cold Spring Harb. Perspect. Med.* **2012**, 2, a006361. [CrossRef] [PubMed]

235. Zare-Shahabadi, A.; Masliah, E.; Johnson, G.V.; Rezaei, N. Autophagy in Alzheimer’s disease. *Rev. Neurosci.* **2015**, 26, 385–395. [CrossRef] [PubMed]

236. Yoon, S.Y.; Kim, D.H. Alzheimer’s disease genes and autophagy. *Brain Res.* **2016**, 1649, 201–209. [CrossRef] [PubMed]

237. Tai, H.; Wang, Z.; Gong, H.; Han, X.; Zhou, J.; Wang, X.; Wei, X.; Ding, Y.; Huang, N.; Qin, J.; et al. Autophagy impairment with lysosomal and mitochondrial dysfunction is an important characteristic of oxidative stress-induced senescence. *Autophagy* **2017**, 13, 99–113. [CrossRef] [PubMed]

238. Cadonic, C.; Sabbir, M.G.; Albens, B.C. Mechanisms of Mitochondrial Dysfunction in Alzheimer’s Disease. *Mol. Neurobiol.* **2016**, 53, 6078–6090. [CrossRef] [PubMed]

239. Gao, J.; Wang, L.; Liu, J.; Xie, F.; Su, B.; Wang, X. Abnormalities of Mitochondrial Dynamics in Neurodegenerative Diseases. *Antioxid. (Basel, Switz.)* **2017**, 6, 25. [CrossRef] [PubMed]

240. Chun, H.; Lee, C.J. Reactive astrocytes in Alzheimer’s disease: A double-edged sword. *Neurosci. Res.* **2018**, 126, 44–52. [CrossRef] [PubMed]

241. Streit, W.J. Microglia and Alzheimer’s disease pathogenesis. *J. Neurosci. Res.* **2004**, 77, 1–8. [CrossRef] [PubMed]

242. Mosher, K.I.; Wyss-Coray, T. Microglial dysfunction in brain aging and Alzheimer’s disease. *Biochem. Pharmacol.* **2014**, 88, 594–604. [CrossRef] [PubMed]
244. Caldeira, C.; Oliveira, A.F.; Cunha, C.; Vaz, A.R.; Falcão, A.S.; Fernandes, A.; Brites, D. Microglia change from a reactive to an age-like phenotype with the time in culture. *Front. Cell. Neurosci.* 2014, 8, 152. [CrossRef] [PubMed]

245. Caldeira, C.; Cunha, C.; Vaz, A.R.; Falcão, A.S.; Barateiro, A.; Seixas, E.; Fernandes, A.; Brites, D. Key aging-associated alterations in primary microglia response to beta-amyloid stimulation. *Front. Aging Neurosci.* 2017, 9, 1–23. [CrossRef] [PubMed]

246. Eitan, E.; Hutchison, E.R.; Mattson, M.P. Telomere shortening in neurological disorders: An abundance of unanswered questions. *Trends Neurosci.* 2014, 37, 256–263. [CrossRef] [PubMed]

247. Boccardi, V.; Pelini, L.; Ercolini, S.; Ruggiero, C.; Meccoci, P. From cellular senescence to Alzheimer’s disease: The role of telomere shortening. *Aging Res. Rev.* 2015, 22, 1–8. [CrossRef] [PubMed]

248. Forero, D.A.; González-Giraldo, Y.; López-Quintero, C.; Castro-Vega, L.J.; Barreto, G.E.; Perry, G.; Kritchevsky, S. Meta-analysis of Telomere Length in Alzheimer’s Disease. *J. Gerontol. A Biol. Sci. Med. Sci.* 2016, 71, 1069–1073. [CrossRef] [PubMed]

249. Hinterberger, M.; Fischer, P.; Huber, K.; Krugluger, W.; Zehetmayer, S. Leukocyte telomere length is linked to vascular risk factors not to Alzheimer’s disease in the VITA study. *J. Neural Transm.* 2017, 124, 809–819. [CrossRef] [PubMed]

250. Jordan-Sciutto, K.L.; Dorsey, R.; Chalovich, E.M.; Hammond, R.R.; Achim, C.L. Expression patterns of retinoblastoma protein in Parkinson disease. *J. Neuropathol. Exp. Neurol.* 2003, 62, 68–74. [CrossRef] [PubMed]

251. Hoglinger, G.U.; Breunig, J.J.; Depboylu, C.; Rouaux, C.; Michel, P.P.; Alvarez-Fischer, D.; Boutillier, A.-L.; DeGregori, J.; Oertel, W.H.; Rakic, P.; et al. The pRb/E2F cell-cycle pathway mediates cell death in Parkinson’s disease. *Proc. Natl. Acad. Sci. USA* 2007, 104, 3585–3590. [CrossRef] [PubMed]

252. van Dijk, K.D.; Persichetti, E.; Chiasserini, D.; Eusebi, P.; Beccari, T.; Calabresi, P.; Berendse, H.W.; Parnetti, L.; Hoglinger, G.U.; Breunig, J.J.; Depboylu, C.; Rouaux, C.; Michel, P.P.; et al. The pRb/E2F cell-cycle pathway mediates cell death in Parkinson’s disease. *Proc. Natl. Acad. Sci. USA* 2007, 104, 3585–3590. [CrossRef] [PubMed]

253. Mogi, M.; Harada, M.; Koné, T.; Riederer, P.; Inagaki, H.; Minami, M.; Nagatsu, T. Interleukin-1β, interleukin-6, epidermal growth factor and transforming growth factor-β are elevated in the brain from parkinsonian patients. *Neurosci. Lett.* 1994, 180, 147–150. [CrossRef]

254. Lindqvist, D.; Kaufman, E.; Brundin, L.; Hall, S.; Surova, Y.; Hansson, O. Non-Motor Symptoms in Patients with Parkinson’s Disease—Correlations with Inflammatory Cytokines in Serum. *PLoS ONE* 2012, 7, e47387. [CrossRef] [PubMed]

255. Brodacki, B.; Staszewski, J.; Toczyłowska, B.; Kozłowska, E.; Drela, N.; Chalimoniuk, M.; Stepien, A. Serum interleukin (IL-2, IL-10, IL-6, IL-4), TNFα, and INFγ concentrations are elevated in patients with atypical and idiopathic parkinsonism. *Neurosci. Lett.* 2008, 441, 158–162. [CrossRef] [PubMed]

256. Scalzo, P.; Kümmer, A.; Cardoso, F.; Teixeira, A.L. Serum levels of interleukin-6 are elevated in patients with Parkinson’s disease and correlate with physical performance. *Neurosci. Lett.* 2010, 468, 56–58. [CrossRef] [PubMed]

257. Hofmann, K.W.; Schuh, A.F.S.; Saute, J.; Townsend, R.; Fricke, D.; Leke, R.; Souza, D.O.; Portela, L.V.; Chaves, M.L.F.; Rieder, C.R.M. Interleukin-6 serum levels in patients with parkinson’s disease. *Neurochem. Res.* 2009, 34, 1401–1404. [CrossRef] [PubMed]

258. Mogi, M.; Harada, M.; Riederer, P.; Narabayashi, H.; Fujita, K.; Nagatsu, T. Tumor necrosis factor-α increases both in the brain and in the cerebrospinal fluid from parkinsonian patients. *Neurosci. Lett.* 1994, 165, 208–210. [CrossRef]

259. Choi, D.-H.; Kim, Y.-J.; Kim, Y.-G.; Joh, T.H.; Beal, M.F.; Kim, Y.-S. Role of Matrix Metalloproteinase 3-mediated a-Synuclein Cleavage in Dopaminergic Cell Death. *J. Biol. Chem.* 2011, 286, 14168–14177. [CrossRef] [PubMed]

260. Kaur, K.; Gill, J.S.; Bansal, P.K.; Deshmukh, R. Neuroinflammation—A major cause for striatal dopaminergic degeneration in Parkinson’s disease. *J. Neuro. Sci.* 2017, 381, 308–314. [CrossRef] [PubMed]

261. Park, J.-S.; Davis, R.L.; Sue, C.M. Mitochondrial Dysfunction in Parkinson’s Disease: New Mechanistic Insights and Therapeutic Perspectives. *Curr. Neurol. Neurosci. Rep.* 2018, 18, 21. [CrossRef] [PubMed]

262. Pitcairn, C.; Wani, W.Y.; Mazzulli, J.R. Dysregulation of the autophagic-lysosomal pathway in Gaucher and Parkinson’s disease. *Neurobiol. Dis.* 2018. [CrossRef] [PubMed]
263. Rivero-Ríos, P.; Madero-Pérez, J.; Fernández, B.; Hilfiker, S. Targeting the Autophagy/Lysosomal Degradation Pathway in Parkinson’s Disease. *Curr. Neuropharmacol.* 2016, 14, 238–249. [CrossRef] [PubMed]

264. Plotegher, N.; Duchen, M.R. Crosstalk between Lysosomes and Mitochondria in Parkinson’s Disease. *Front. Cell Dev. Biol.* 2017, 5, 110. [CrossRef] [PubMed]

265. Wafaa, G.; Dragonas, C.; Brosche, T.; Dittrich, R.; Sieber, C.C.; Alcuc, E.; Benetos, A.; Nzietchueng, R. Study of telomere length and different markers of oxidative stress in patients with Parkinson’s disease. *J. Nutr. Health Aging* 2011, 15, 277–281. [CrossRef] [PubMed]

266. Maeda, T.; Guan, J.Z.; Koyanagi, M.; Higuchi, Y.; Makino, N. Aging-Associated Alteration of Telomere Length and Subtelomeric Status in Parkinson’s Patients’ Disease. *J. Neurogenet.* 2012, 26, 245–251. [CrossRef] [PubMed]

267. Guan, J.Z.; Maeda, T.; Sugano, M.; Oyama, J.; Higuchi, Y.; Suzuki, T.; Makino, N. A percentage analysis of the telomere length in Parkinson’s disease patients. *J. Gerontol. A Biol. Sci. Med. Sci.* 2008, 63, 467–473. [CrossRef] [PubMed]

268. Eerola, J.; Kananen, L.; Manninen, K.; Hellstrom, O.; Tienari, P.J.; Hovatta, I. No Evidence for Shorter Leukocyte Telomere Length in Parkinson’s Disease Patients. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* 2010, 65A, 1181–1184. [CrossRef] [PubMed]

269. Degerman, S.; Domellöf, M.; Landfors, M.; Linder, J.; Lundin, M.; Haraldsson, S.; Elgh, E.; Roos, G.; Forsgren, L. Long leukocyte telomere length at diagnosis is a risk factor for dementia progression in idiopathic parkinsonism. *PLoS ONE* 2014, 9, e113387. [CrossRef] [PubMed]

270. Forero, D.A.; González-Giraldo, Y.; López-Quintero, C.; Castro-Vega, L.J.; Barreto, G.E.; Perry, G. Telomere length in Parkinson’s disease: A meta-analysis HHS Public Access. *Exp. Gerontol.* 2016, 75, 53–55. [CrossRef] [PubMed]

271. Compston, A.; Coles, A. Multiple sclerosis. *Lancet* 2008, 372, 1502–1517. [CrossRef]

272. Ebers, G.C. Environmental factors and multiple sclerosis. *Multiple sclerosis*. 2008, 14, 268–277. [CrossRef]

273. Pardo, G.; Jones, D.E. The sequence of disease-modifying therapies in relapsing multiple sclerosis: Safety and immunologic considerations. *J. Neurol.* 2017, 264, 2351–2374. [CrossRef] [PubMed]

274. Papadopoulos, D.; Pham-Dinh, D.; Reynolds, R. Axon loss is responsible for chronic neurological deficit following inflammatory demyelination in the rat. *Exp. Neurol.* 2006, 197, 373–385. [CrossRef] [PubMed]

275. Papadopoulos, D.; Dukes, S.; Patel, R.; Nicholas, R.; Vora, A.; Reynolds, R. Substantial Archaeocortical Atrophy and Neuronal Loss in Multiple Sclerosis. *Brain Pathol.* 2009, 19, 238–253. [CrossRef] [PubMed]

276. Lassmann, H.; Brück, W.; Lucchinetti, C.F. The Immunopathology of Multiple Sclerosis: An Overview. *Brain Pathol.* 2007, 17, 210–218. [CrossRef] [PubMed]

277. Gilgun-Sherki, Y.; Melamed, E.; Offen, D. The role of oxidative stress in the pathogenesis of multiple sclerosis: The need for effective antioxidant therapy. *J. Neurol.* 2004, 251, 261–268. [PubMed]

278. Mahad, D.; Lassmann, H.; Turnbull, D. Mitochondria and disease progression in multiple sclerosis. *Neuropathol. Appl. Neurobiol.* 2008, 34, 577–589. [CrossRef] [PubMed]

279. Mahad, D.; Ziabreva, I.; Lassmann, H.; Turnbull, D. Mitochondrial defects in acute multiple sclerosis lesions. *Brain* 2008, 131, 1722–1735. [CrossRef] [PubMed]

280. Mahad, D.J.; Ziabreva, I.; Campbell, G.; Lax, N.; White, K.; Hanson, P.S.; Lassmann, H.; Turnbull, D.M. Mitochondrial changes within axons in multiple sclerosis. *Brain* 2009, 132, 1161–1174. [CrossRef] [PubMed]

281. Bergamaschi, R.; Quaglini, S.; Tavazzi, E.; Amato, M.P.; Paolicelli, D.; Zipoli, V.; Romani, A.; Tortorella, C.; Portaccio, E.; D’Onghia, M.; et al. Immunomodulatory therapies delay disease progression in multiple sclerosis. *Mult. Scler. J.* 2016, 22, 1732–1740. [CrossRef] [PubMed]

282. Karamita, M.; Nicholas, R.; Kokoti, L.; Rizou, S.; Mitsikostas, D.D.; Gorgoulis, V.; Probert, L.; Papadopoulos, D. Cellular Senescence Correlates with Demyelination, Brain Atrophy and Motor Impairment in a Model of Multiple Sclerosis (P2.405). *Neurology* 2018, 90, no 15 supplement.

283. Bø, L.; Vedeler, C.A.; Nyland, H.I.; Trapp, B.D.; Mørk, S.J. Subpial demyelination in the cerebral cortex of multiple sclerosis patients. *J. Neuropathol. Exp. Neurol.* 2003, 62, 723–732. [CrossRef] [PubMed]

284. Calabrese, M.; Filippi, M.; Gallo, P. Cortical lesions in multiple sclerosis. *Nat. Rev. Neurol.* 2010, 6, 438–444. [CrossRef] [PubMed]

285. Magliozi, R.; Howell, O.W.; Reeves, C.; Roncaroli, F.; Nicholas, R.; Serafini, B.; Aloisi, F.; Reynolds, R. A Gradient of neuronal loss and meningeal inflammation in multiple sclerosis. *Ann. Neurol.* 2010, 68, 477–493. [CrossRef] [PubMed]
286. Peterson, J.W.; Bö, L.; Mörk, S.; Chang, A.; Trapp, B.D. Transected neurites, apoptotic neurons, and reduced inflammation in cortical multiple sclerosis lesions. Ann. Neurol. 2001, 50, 389–400. [CrossRef] [PubMed]
287. Trapp, B.D.; Peterson, J.; Ransohoff, R.M.; Rudick, R.; Mörk, S.; Bö, L. Axonal transection in the lesions of multiple sclerosis. N. Engl. J. Med. 1998, 338, 278–285. [CrossRef] [PubMed]
288. Anderson, R.M.; Hadjichrysanthou, C.; Evans, S.; Wong, M.M. Why do so many clinical trials of therapies for Alzheimer’s disease fail? Lancet 2017, 390, 2327–2329. [CrossRef]
289. Cummings, J. Lessons Learned from Alzheimer Disease: Clinical Trials with Negative Outcomes. Clin. Transl. Sci. 2018, 11, 147–152. [CrossRef] [PubMed]
290. Marchant, N.L.; Reed, B.R.; DeCarli, C.S.; Madison, C.M.; Weiner, M.W.; Chui, H.C.; Jagust, W.J. Cerebrovascular disease, β-amyloid, and cognition in aging. Neurobiol. Aging 2012, 33. [CrossRef] [PubMed]
291. Bartzokis, G. Alzheimer’s disease as homeostatic responses to age-related myelin breakdown. Neurobiol. Aging 2011, 32, 1341–1371. [CrossRef] [PubMed]
292. Ossenkoppele, R.; Jansen, W.J.; Rabinovici, G.D.; Knol, D.L.; van der Flier, W.M.; van Berckel, B.N.M.; Scheltens, P.; Visser, P.J.; Verfaillie, S.C.J.; Zwan, M.D.; et al. Prevalence of Amyloid PET Positivity in Dementia Syndromes. JAMA 2015, 313, 1939–1949. [CrossRef] [PubMed]
293. Jansen, W.J.; Ossenkoppele, R.; Knol, D.L.; Tijms, B.M.; Scheltens, P.; Verhey, F.R.J.; Visser, P.J.; Aalten, P.; Aarsland, D.; Alcolea, D.; et al. Prevalence of Cerebral Amyloid Pathology in Persons Without Dementia. JAMA 2015, 313, 1924–1938. [CrossRef] [PubMed]
294. Dickson, M.A.; Tap, W.D.; Keohan, M.L.; D’Angelo, S.P.; Antonescu, C.R.; Landa, J.; Qin, L.-X.; Rathbone, D.D.; Condy, M.M.; et al. Phase II trial of the CDK4 inhibitor PD0332991 in patients with advanced CDK4-amplified well-differentiated or dedifferentiated liposarcoma. J. Clin. Oncol. 2013, 31, 2024–2028. [CrossRef] [PubMed]
295. Jun, J.-I.; Lau, L.F. Cellular senescence controls fibrosis in wound healing. Aging (Albany NY) 2010, 2, 627–631. [CrossRef] [PubMed]
296. DiRocco, D.P.; Kobayashi, A.; Taketo, M.M.; McMahon, A.P.; Humphreys, B.D. Wnt4/β-Catenin Signaling in Medullary Kidney Myofibroblasts. J. Am. Soc. Nephrol. 2013, 24, 1399–1412. [CrossRef] [PubMed]
297. Kim, Y.-M.; Byun, H.-O.; Lee, B.A.; Cho, H.; Seo, Y.-H.; Kim, Y.-S.; Park, M.H.; Chung, H.-Y.; Woo, H.G.; Yoon, G. Implications of time-series gene expression profiles of replicative senescence. Aging Cell 2013, 12, 622–634. [CrossRef] [PubMed]
298. Kong, X.; Feng, D.; Wang, H.; Hong, F.; Bertola, A.; Wang, F.-S.; Gao, B. Interleukin-22 induces hepatic stellate cell senescence and restricts liver fibrosis in mice. Hepatology 2012, 56, 1150–1159. [CrossRef] [PubMed]
299. Borkham-Kamphorst, E.; Schaffrath, C.; Van de Leur, E.; Haas, U.; Thaa, L.; Meurer, S.K.; Nevzorova, Y.A.; Liedtke, C.; Weiskirchen, R. The anti-fibrotic effects of CCN1/CYR61 in primary portal myofibroblasts are mediated through induction of reactive oxygen species resulting in cellular senescence, apoptosis and attenuated TGF-β signaling. Biochem. Biophys. Acta Mol. Cell Res. 2014, 1843, 902–914. [CrossRef] [PubMed]
300. de la Fuente-Fernández, R.; Schulzer, M.; Kuramoto, L.; Cragg, J.; Ramachandiran, N.; Au, W.L.; Mak, E.; McKenzie, J.; McCormick, S.; Sossi, V.; et al. Age-specific progression of nigrostriatal dysfunction in Parkinson’s disease. Ann. Neurol. 2011, 69, 803–810. [CrossRef] [PubMed]
301. Myrianthopoulos, V.; Evangelou, K.; Vasileiou, P.V.S.; Cooks, T.; Vassilakopoulos, T.P.; Pangalis, G.A.; Kouloukoussa, M.; Kittas, C.; Georgakilas, A.G.; Gorgoulis, V.G. Senescence and senotherapeutics: A new field in cancer therapy. Pharmacol. Ther. 2018. [CrossRef] [PubMed]
302. Ng, T.P.; Feng, L.; Yap, K.B.; Lee, T.S.; Tan, C.H.; Winblad, B. Long-Term Metformin Usage and Cognitive Function among Older Adults with Diabetes. J. Alzheimer’s Dis. 2014, 41, 61–68. [CrossRef] [PubMed]
303. Kuan, Y.-C.; Huang, K.-W.; Lin, C.-L.; Hu, C.-J.; Kao, C.-H. Effects of metformin exposure on neurodegenerative diseases in elderly patients with type 2 diabetes mellitus. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 2017, 79, 77–83. [CrossRef] [PubMed]
306. Althubiti, M.; Lezina, L.; Carrera, S.; Jukes-Jones, R.; Giblett, S.M.; Antonov, A.; Barlev, N.; Saldanha, G.S.; Pritchard, C.A.; Cain, K.; et al. Characterization of novel markers of senescence and their prognostic potential in cancer. *Cell Death Dis.* **2014**, *5*, e1528. [CrossRef] [PubMed]

307. As, J.; Chen, P.; Norris, D.; Padmanabha, R.; Lin, J.; Moquin, R.V.; Shen, Z.; Cook, L.S.; Doweyko, A.M.; Pitt, S.; et al. 2-Aminothiazole as a Novel Kinase Inhibitor Template. Structure—Activity Relationship Studies toward the Discovery of N-(2-Chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-1,3-thiazole-5-carboxamide (Dasatinib, BMS-354825) as a potent pan-Src Kinase Inhibitor. *J. Med. Chem.* **2006**, *49*, 6819–6832.

308. Han, L.; Schuringa, J.J.; Mulder, A.; Vellenga, E. Dasatinib impairs long-term expansion of leukemic progenitors in a subset of acute myeloid leukemia cases. *Ann. Hematol.* **2010**, *89*, 861–871. [CrossRef] [PubMed]

309. Keating, G.M. Dasatinib: A Review in Chronic Myeloid Leukaemia and Ph+ Acute Lymphoblastic Leukaemia. *Drugs* **2017**, *77*, 85–96. [CrossRef] [PubMed]

310. Li, J.; Rix, U.; Fang, B.; Bai, Y.; Edwards, A.; Colinge, J.; Bennett, K.L.; Gao, J.; Song, L.; Eschrich, S.; et al. A chemical and phosphoproteomic characterization of dasatinib action in lung cancer. *Nat. Chem. Biol.* **2010**, *6*, 291–299. [CrossRef] [PubMed]

311. Ay, M.; Charli, A.; Jin, H.; Anantharam, V.; Kanthasamy, A.; Kanthasamy, A.G. Quercetin. In *Nutraceuticals*; Gupta, R.C., Ed.; Academic Press: Boston, MA, USA, 2016; pp. 447–452.

312. Jaffe, R.; Mani, J. Polyphenolics Evoke Healing Responses: Clinical Evidence and Role of Predictive Biomarkers. In *Polyphenols in Human Health and Disease*; Watson, R.R., Preedy, V.R., Sherma, Z., Eds.; Academic Press: San Diego, CA, USA, 2014; pp. 695–705.

313. Middleton, E.; Kandaswami, C.; Theoharides, T.C. The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. *Pharmacol. Rev.* **2000**, *52*, 673–751. [PubMed]

314. Wang, W.; Sun, C.; Mao, L.; Ma, P.; Liu, F.; Yang, J.; Gao, Y. The biological activities, chemical stability, metabolism and delivery systems of quercetin: A review. *Trends Food Sci. Technol.* **2016**, *56*, 21–38. [CrossRef]

315. Shankar, G.M.; Antony, J.; Anto, R.J. Quercetin and Tryptanthrin: Two Broad Spectrum Anticancer Agents for Future Chemotherapeutic Interventions. In *The Enzymes*; Bathaie, S.Z., Tamanoi, F., Eds.; Academic Press: Cambridge, MA, USA, 2015; Volume 37, pp. 43–72.

316. Schnekenburger, M.; Diederich, M. Nutritional Epigenetic Regulators in the Field of Cancer: New Avenues for Chemopreventive Approaches. In *Epigenetic Cancer Therapy*; Gray, S.G., Ed.; Academic Press: Boston, MA, USA, 2015; pp. 393–425.

317. Hubbard, G.P.; Stevens, J.M.; Cicmil, M.; Sage, T.; Jordan, P.A.; Williams, C.M.; Lovegrove, J.A.; Gibbins, J.M. Quercetin inhibits collagen-stimulated platelet activation through inhibition of multiple components of the glycoprotein VI signaling pathway. *J. Thromb. Haemost.* **2003**, *1*, 1079–1088. [CrossRef] [PubMed]

318. Hubbard, G.P.; Wolfmam, S.; Lovegrove, J.A.; Gibbins, J.M. Ingestion of quercetin inhibits platelet aggregation and essential components of the collagen-stimulated platelet activation pathway in humans. *J. Thromb. Haemost.* **2004**, *2*, 2138–2145. [CrossRef] [PubMed]

319. Feitelson, M.A.; Arzumanany, A.; Kulathinal, R.J.; Blain, S.W.; Holcombe, R.F.; Mahajna, J.; Marino, M.; Martinez-Chantar, M.L.; Nawroth, R.; Sanchez-Garcia, I.; et al. Sustained proliferation in cancer: Mechanisms and novel therapeutic targets. *Semin. Cancer Biol.* **2015**, *35*, S25–S54. [CrossRef] [PubMed]

320. Williams, R.J.; Spencer, J.P.; Rice-Evans, C. Flavonoids: Antioxidants or signalling molecules? *Free Radic. Biol. Med.* **2004**, *36*, 838–849. [CrossRef] [PubMed]

321. Russo, G.L.; Russo, M.; Spagnuolo, C.; Tedesco, I.; Bilotto, S.; Iannitti, R.; Palumbo, R. Quercetin: A Pleiotropic Kinase Inhibitor Against Cancer BT—Advances in Nutrition and Cancer; Zappia, V., Panicò, S., Russo, G.L., Budillon, A., Della Ragione, F., Eds.; Springer: Berlin/Heidelberg, Germany, 2014; pp. 185–205.

322. Maggioni, M.; Vivacqua, A.; Fasanella, G.; Recchia, A.G.; Sisci, D.; Pezzi, V.; Montanaro, D.; Musti, A.M.; Picard, D.; Andò, S. The G Protein-coupled Receptor GPR30 Mediates ε-fos up-regulation by 17-β-Estradiol and Phytoestrogens in Breast Cancer Cells. *J. Biol. Chem.* **2004**, *279*, 27008–27016. [CrossRef] [PubMed]

323. Zhu, Y.; Tchkonia, T.; Pirskkalava, T.; Gower, A.C.; Ding, H.; Giorgadze, N.; Palmer, A.K.; Ikeno, Y.; Hubbard, G.B.; Lenburg, M.; et al. The Achilles’ heel of senescent cells: From transcriptome to senolytic drugs. *Aging Cell* **2015**, *14*, 644–658. [CrossRef] [PubMed]
324. Zhu, Y.; Tchkonia, T.; Fuhrmann-Stroissnigg, H.; Dai, H.M.; Ling, Y.Y.; Stout, M.B.; Pirtskhalava, T.; Giorgadze, N.; Johnson, K.O.; Giles, C.B.; et al. Identification of a novel senolytic agent, navitoclax, targeting the Bcl-2 family of anti-apoptotic factors. Aging Cell 2016, 15, 428–435. [CrossRef] [PubMed]

325. Chang, J.; Wang, Y.; Shao, L.; Laberge, R.-M.; Demaria, M.; Campisi, J.; Janakiraman, K.; Sharpless, N.E.; Ding, S.; Feng, W.; et al. Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. Nat. Med. 2016, 22, 78–83. [CrossRef] [PubMed]

326. Tse, C.; Shoemaker, A.R.; Adickes, J.; Anderson, M.G.; Chen, J.; Jin, S.; Johnson, E.F.; Marsh, K.C.; Mitten, M.J.; Nimmer, P.; et al. ABT-263: A Potent and Orally Bioavailable Bcl-2 Family Inhibitor. Cancer Res. 2008, 68, 3421–3428. [CrossRef] [PubMed]

327. Garland, W.; Benezra, R.; Chaudhary, J. Targeting Protein–Protein Interactions to Treat Cancer—Recent Progress and Future Directions. In Annual Reports in Medicinal Chemistry; Desai, M.C., Ed.; Academic Press: Cambridge, MA, USA, 2013; Volume 48, pp. 227–245.

328. Kim, H.-N.; Chang, J.; Shao, L.; Han, L.; Iyer, S.; Manolagas, S.C.; O’Brien, C.A.; Jilka, R.L.; Zhou, D.; Almeida, M. DNA damage and senescence in osteoprogenitors expressing Osx1 may cause their decrease with age. Aging Cell 2017, 16, 693–703. [CrossRef] [PubMed]

329. Pan, J.; Li, D.; Xu, Y.; Zhang, J.; Chen, M.; Lin, S.; Huang, L.; Chung, E.J.; Citrin, D.E.; et al. Inhibition of Bcl-2/xl With ABT-263 Selectively Kills Senescent Type II Pneumocytes and Reverses Persistent Pulmonary Fibrosis Induced by Ionizing Radiation in Mice. Int. J. Radiat. Oncol. 2017, 99, 353–361. [CrossRef] [PubMed]

330. Demaria, M.; O’Leary, M.N.; Chang, J.; Shao, L.; Liu, S.; Alimirah, F.; Koenig, K.; Le, C.; Mitin, N.; Deal, A.M.; et al. Cellular Senescence Promotes Adverse Effects of Chemotherapy and Cancer Relapse. Cancer Discov. 2017, 7, 165–176. [CrossRef] [PubMed]

331. Yosef, R.; Pilpel, N.; Tokarsky-Amiel, R.; Biran, A.; Ovadya, Y.; Cohen, S.; Vadai, E.; Dassa, L.; Shahar, E.; Conditti, R.; et al. Directed elimination of senescent cells by inhibition of BCL-W and BCL-XL. Nat. Commun. 2016, 7, 11190. [CrossRef] [PubMed]

332. Del Gaizo Moore, V.; Brown, J.R.; Certo, M.; Love, T.M.; Novina, C.D.; Letai, A. Chronic lymphocytic leukemia requires BCL2 to sequester prodeath BIM, explaining sensitivity to BCL2 antagonist ABT-737. J. Clin. Invest. 2007, 117, 112–121. [CrossRef] [PubMed]

333. van Delft, M.F.; Wei, A.H.; Mason, K.D.; Vandenberg, C.J.; Chen, L.; Czabotar, P.E.; Willis, S.N.; Scott, C.L.; Day, C.L.; Cory, S.; et al. The BH3 mimetic ABT-737 targets selective Bcl-2 proteins and efficiently induces apoptosis via Bak/Bax if Mcl-1 is neutralized. Cancer Cell 2006, 10, 389–399. [CrossRef] [PubMed]

334. Konopleva, M.; Contractor, R.; Tsao, T.; Samudio, I.; Ruvolore, P.P.; Kitada, S.; Deng, X.; Zhai, D.; Shi, Y.-X.; Sneed, T.; et al. Mechanisms of apoptosis sensitivity and resistance to the BH3 mimetic ABT-737 in acute myeloid leukemia. Cancer Cell 2006, 10, 375–388. [CrossRef] [PubMed]

335. Zhu, Y.; Doornebal, E.J.; Pirtskhalava, T.; Giorgadze, N.; Wentworth, M.; Fuhrmann-Stroissnigg, H.; Niedernhofer, L.J.; Robbins, P.D.; Tchkonia, T.; Kirkland, J.L. New agents that target senescent cells: The flavone, fisetin, and the BCL-XL inhibitors, A1331852 and A1155463. Aging (Albany NY) 2017, 9, 955–963. [CrossRef] [PubMed]

336. Maher, P. Fisetin Acts on Multiple Pathways to Reduce the Impact of Age and Disease on CNS Function. Front. Biosci. (Schol. Ed.) 2015, 7, 58–82. [CrossRef] [PubMed]

337. Naeimi, A.F.; Alizadeh, M. Antioxidant properties of the flavonoid fisetin: An updated review of in vivo and in vitro studies. Trends Food Sci. Technol. 2017, 70, 34–44. [CrossRef]

338. Mathers, J.C.; Stratheen, G.; Relton, C.L. Induction of Epigenetic Alterations by Dietary and Other Environmental Factors. In Advances in Genetics; Herceg, Z., Ushijima, T., Eds.; Academic Press: Cambridge, MA, USA, 2010; Volume 71, pp. 3–39.

339. Kim, J.; Lee, K.W.; Lee, H.J. Polyphenols Suppress and Modulate Inflammation: Possible Roles in Health and Disease. In Polyphenols in Human Health and Disease; Academic Press: San Diego, CA, USA, 2014; pp. 393–408.

340. Esselen, M.; Barth, S.W. Food-Borne Topoisomerase Inhibitors: Risk or Benefit. In Advances in Molecular Toxicology; Fishbein, J.C., Heilman, J.M., Eds.; Elsevier: New York, NY, USA, 2014; Volume 8, pp. 123–171.

341. Adhami, V.M.; Syed, D.N.; Khan, N.; Mukhtar, H. Dietary flavonoid fisetin: A novel dual inhibitor of PI3K/Akt and mTOR for prostate cancer management. Biochem. Pharmacol. 2012, 84, 1277–1281. [CrossRef] [PubMed]
342. Lu, H.; Chang, D.J.; Baratte, B.; Meijer, L.; Schulze-Gahmen, U. Crystal Structure of a Human Cyclin-Dependent Kinase 6 Complex with a Flavonol Inhibitor, Fisetin. *J. Med. Chem.* 2005, 48, 737–743. [CrossRef] [PubMed]

343. Webb, M.R.; Ebeler, S.E. Comparative analysis of topoisomerase IB inhibition and DNA intercalation by flavonoids and similar compounds: Structural determinates of activity. *Biochem. J.* 2004, 384, 527–541. [CrossRef] [PubMed]

344. Syed, D.N.; Adhami, V.M.; Khan, M.I.; Mukhtar, H. Inhibition of Akt/mTOR signaling by the dietary flavonoid fisetin. *Anticancer Agents Med. Chem.* 2013, 13, 995–1001. [CrossRef] [PubMed]

345. Gupta, S.C.; Tyagi, A.K.; Hinojosa, M.; Prasad, S.; Aggarwal, B.B. Downregulation of tumor necrosis factor and other proinflammatory biomarkers by polyphenols. *Arch. Biochem. Biophys.* 2014, 559, 91–99. [CrossRef] [PubMed]

346. Khan, N.; Syed, D.N.; Ahmad, N.; Mukhtar, H. Fisetin: A Dietary Antioxidant for Health Promotion. *Antioxid. Redox Signal.* 2013, 19, 151–162. [CrossRef] [PubMed]

347. Wang, Y.; Chang, J.; Liu, X.; Zhang, X.; Zhang, S.; Zhang, X.; Zhou, D.; Zheng, G. Discovery of piperlongumine as a potential novel lead for the development of senolytic agents. *Aging (Albany NY)* 2016, 8, 2915–2926. [CrossRef] [PubMed]

348. Zheng, J.; Son, D.J.; Gu, S.M.; Woo, J.R.; Ham, Y.W.; Lee, H.P.; Kim, W.J.; Jung, J.K.; Hong, J.T. Piperlongumine inhibits lung tumor growth via inhibition of nuclear factor kappa B signaling pathway. *Sci. Rep.* 2016, 6, 26357. [CrossRef] [PubMed]

349. Fuhrmann-Stroissnigg, H.; Ling, Y.Y.; Zhao, J.; McGowan, S.J.; Zhu, Y.; Brooks, R.W.; Grassi, D.; Gregg, S.Q.; Striay, J.L.; Dorronsoro, A.; et al. Identification of HSP90 inhibitors as a novel class of senolytics. *Nat. Commun.* 2017, 8, 422. [CrossRef] [PubMed]

350. Roe, S.M.; Prodromou, C.; O’Brien, R.; Ladbury, J.E.; Piper, P.W.; Pearl, L.H. Structural Basis for Inhibition of the Hsp90 Molecular Chaperone by the Antitumor Antibiotics Radicicol and Geldanamycin. *J. Med. Chem.* 1999, 42, 260–266. [CrossRef] [PubMed]

351. Samaraweera, L.; Adomako, A.; Rodriguez-Gabin, A.; McDaid, H.M. A Novel Indication for Panobinostat as a Senolytic Drug in NSCLC and HNSCC. *Sci. Rep.* 2017, 7, 1900. [CrossRef] [PubMed]

352. Lim, H.; Park, H.; Kim, H.P. Effects of flavonoids on senescence-associated secretory phenotype formation from bleomycin-induced senescence in BJ fibroblasts. *Biochem. Pharmacol.* 2015, 96, 337–348. [CrossRef] [PubMed]

353. Ballou, L.M.; Lin, R.Z. Rapamycin and mTOR kinase inhibitors. *J. Chem. Biol.* 2008, 1, 27–36. [CrossRef] [PubMed]

354. Pospelova, T.V.; Leontieva, O.V.; Bykova, T.V.; Zubova, S.G.; Pospelov, V.A.; Blagosklonny, M.V. Suppression of replicative senescence by rapamycin in rodent embryonic cells. *Cell Cycle* 2012, 11, 2402–2407. [CrossRef] [PubMed]

355. Li, J.; Kim, S.G.; Blenis, J. Rapamycin: One Drug, Many Effects. *Cell Metab.* 2014, 19, 373–379. [CrossRef] [PubMed]

356. Arriola Apelo, S.I.; Lamming, D.W. Rapamycin: An InhibiTOR of Aging Emerges From the Soil of Easter Island. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* 2016, 71, 841–849. [CrossRef] [PubMed]

357. Xu, M.; Tchekhova, T.; Ding, H.; Ogrodnik, M.; Lubbers, E.R.; Pirtskhalava, T.; White, T.A.; Johnson, K.O.; Stout, M.B.; Mezera, V.; et al. JAK inhibition alleviates the cellular senescence-associated secretory phenotype and frailty in old age. *Proc. Natl. Acad. Sci. USA* 2015, 112, E6301–E6310. [CrossRef] [PubMed]

358. Moiseeva, O.; Deschênes-Simard, X.; St-Germain, E.; Igelmann, S.; Huot, G.; Cadar, A.E.; Bourdeau, V.; Pollak, M.N.; Ferbeyre, G. Metformin inhibits the senescence-associated secretory phenotype by interfering with IKK/NF-kB activation. *Aging Cell* 2013, 12, 489–498. [CrossRef] [PubMed]

359. Chen, D.; Xia, D.; Pan, Z.; Xu, D.; Zhou, Y.; Wu, Y.; Cai, N.; Tang, Q.; Wang, C.; Yan, M.; et al. Metformin protects against apoptosis and senescence in nucleus pulposus cells and ameliorates disc degeneration in vivo. *Cell Death Dis.* 2016, 7, e2441. [CrossRef] [PubMed]

360. Barzilai, N.R. Targeting aging with metformin (tame). *Innov. Aging* 2017, 743. [CrossRef]

361. Rena, G.; Hardie, D.G.; Pearson, E.R. The mechanisms of action of metformin. *Diabetologia* 2017, 60, 1577–1585. [CrossRef] [PubMed]
362. Laberge, R.-M.; Zhou, L.; Sarantos, M.R.; Rodier, F.; Freund, A.; de Keizer, P.L.J.; Liu, S.; Demaria, M.; Cong, Y.-S.; Kapahi, P.; et al. Glucocorticoids suppress selected components of the senescence-associated secretory phenotype. *Aging Cell* **2012**, *11*, 569–578. [CrossRef] [PubMed]

363. Sinclair, D.A.; Guarente, L. Small-Molecule Allosteric Activators of Sirtuins. *Annu. Rev. Pharmacol. Toxicol.* **2014**, *54*, 363–380. [CrossRef] [PubMed]

364. Hubbard, B.P.; Sinclair, D.A. Small molecule SIRT1 activators for the treatment of aging and age-related diseases. *Trends Pharmacol. Sci.* **2014**, *35*, 146–154. [CrossRef] [PubMed]

365. Borra, M.T.; Smith, B.C.; Denu, J.M. Mechanism of Human SIRT1 Activation by Resveratrol. *J. Biol. Chem.* **2005**, *280*, 17187–17195. [CrossRef] [PubMed]

366. Pitozzi, V.; Mocali, A.; Laurenzana, A.; Giannoni, E.; Cifola, I.; Battaglia, C.; Chiarugi, P.; Dolara, P.; Giovannelli, L. Chronic Resveratrol Treatment Ameliorates Cell Adhesion and Mitigates the Inflammatory Phenotype in Senescent Human Fibroblasts. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* **2013**, *68*, 371–381. [CrossRef] [PubMed]

367. Vassilev, L.T.; Vu, B.T.; Graves, B.; Carvajal, D.; Podlaski, F.; Filipovic, Z.; Kong, N.; Kammlott, U.; Lukacs, C.; Klein, C.; et al. In Vivo Activation of the p53 Pathway by Small-Molecule Antagonists of MDM2. *Science (80-.)* **2004**, *303*, 844–848. [CrossRef] [PubMed]

368. Mouraret, N.; Marcos, E.; Abid, S.; Gary-Bobo, G.; Saker, M.; Houssaini, A.; Dubois-Rande, J.-L.; Boyer, L.; Boczkowski, J.; Derumeaux, G.; et al. Activation of Lung p53 by Nutlin-3a Prevents and Reverses Experimental Pulmonary Hypertension. *Circulation* **2013**, *127*, 1664–1676. [CrossRef] [PubMed]

369. Poulsen, R.C.; Watts, A.C.; Murphy, R.J.; Snelling, S.J.; Carr, A.J.; Hulley, P.A. Glucocorticoids induce senescence in primary human tenocytes by inhibition of sirtuin 1 and activation of the p53/p21 pathway: In vivo and in vitro evidence. *Ann. Rheum. Dis.* **2014**, *73*, 1405–1413. [CrossRef] [PubMed]

370. Moiseeva, O.; Mallette, F.A.; Mukhopadhyay, U.K.; Moores, A.; Ferbeyre, G. DNA damage signaling and p53-dependent senescence after prolonged beta-interferon stimulation. *Mol. Biol. Cell.* **2006**, *17*, 1583–1592. [CrossRef] [PubMed]

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