ASTROCYTE AND MICROGLIA SENESCENCE IN PARKINSON'S DISEASE

BACHELOR THESIS - NEUROSCIENCES RESEARCH

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ABSTRACT

Cellular senescence is a state of permanent cell arrest which is accompanied by multiple phenotypical changes including the secretion of pro-inflammatory mediators. These alterations have both beneficial and deleterious effects on the environment, making the occurrence of senescence important for various biological processes and pathologies. It is only since recently that research started to look at senescence in the CNS and several links with neurodegenerative diseases are already being established. Senescence in astrocytes and microglia may play an important role as their neuroinflammatory properties are known to contribute to neurodegenerative diseases like Parkinson's Disease. In this review, we will try to answer what the molecular basis is behind senescence and how senescence in astrocytes and microglia may play a role in the pathology of Parkinson's disease, emphasizing its contribution to neuroinflammation.

Keywords SASP · Senescence · Astrocyte · Microglia · Parkinson

1 Introduction

The first evidence of cellular senescence came from the cultivation experiments of Hayflick and Moorhead in 1961 and 1965 [1, 2]. In an academic world where cells were believed to be "immortal", Hayflick and Moorhead proved the opposite by showing that their human diploid fibroblast stopped dividing after creating 50 sub-cultivations. Only after decades it was discovered that the shortening of the telomeres was responsible for this "mortality" [3]. These unexpected discoveries led to the start of a whole new field in molecular biology and has now come to the development of new therapeutics for age-related diseases.

Initially, cellular senescence was believed to be nothing more than a stop on the cell-cycle to prevent future damage to the DNA. Interestingly, new evidence showed that senescent cells also change their metabolic pathways, including a key alteration in which the senescent cells starts to secrete different molecules including cytokines, chemokines and proteinases, known as the senescence associated secretory phenotype (SASP) [4, 5]. The SASP is capable of influencing its micro-environment by affecting neighbouring cells and is best known for its pro-inflammatory character. Because senescent cells accumulate with age, it is proposed that the SASP may contribute to the low-level chronic inflammation seen during ageing and can contribute to age-related pathologies [5, 6]. Cellular senescence can be induced by a large number of stimuli including DNA damage, oxidative/oncogenic stress and chromatin disruption rather than only by telomere attrition [7]. Every stimulus is responsible for a different senescent phenotype and every type of cell reacts differently as well, making the overall phenotype very heterogeneous.

However, despite their different stimuli and reaction, senescent cells show general pathways responsible for their phenotype. Signalling molecules from these pathways are currently being used as markers to identify senescent cells in addition to the typical changes found in senescent cells. The identification of senescent cells, by using these markers, showed the presence of senescence in many different cell types including glial cells and neurons [8, 9]. These discoveries started a new chapter in senescence research focused on the involvement of senescence in age-related neurodegeneration and is recently starting to show promising results.

Parkinson disease (PD) is the second most common neurodegenerative disease and is characterized by a number of symptoms including: resting tremor, slowness of movement, rigidity, postural instability and the progressive loss of dopaminergic neurons in the Substantia Nigra pars compacta (SNpc) [10]. The exact cause of PD is still unknown but is very likely linked to the aggregation of α-synuclein in the dopaminergic neurons in the SNpc [11]. Research from the last decade showed that chronic inflammation may play a key role in PD. There is a large amount of evidence supporting the presence of a pro-inflammatory environment including the identification of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-1β [12]. Astrocytes and microglia are capable of secreting these cytokines and, in addition, α-synuclein is capable of inducing this secretion [13, 14].

With the evidence of senescence present in astrocytes and microglia, and their involvement in creating a pro-inflammatory environment in PD, new senescence research is now starting to focus on a possible role for senescence in PD. In this review we will provide an overview of the general pathways involved in creating the phenotype of senescent cells and provide the current evidence for senescence in astrocytes and microglia. Next, we will discuss the role of senescent astrocytes and microglia in the PD pathology, where astrocytes may play a major role. With this information we will try to answer whether there is a role for astrocyte and microglia senescence in the pathology of Parkinson's Disease, emphasizing on the possible contribution to neuroinflammation.

2 Neuroinflammation in Parkinson's Disease

2.1 Astrocytes

Astrocytes are the most abundant glial cells present in the central nervous system (CNS) and are involved in many different functions [15, 16]. They guide the migration of newly developing axons by making boundaries [17], take part in the manipulation of the blood flow to adjust for energy demand [18, 19], are responsible for the glucose, ion, pH and neurotransmitter homeostasis [16], regulate control of synaptic transmission and plasticity [20] and help with the formation of the blood brain barrier and synapses [20, 21].

In response to injury or disease, astrocyte change in a molecular, cellular and functional manner, a process called astrogliosis [22, 23]. It has been proposed that they transform to either the A1 state or the A2 state. A1 is pro-inflammatory and is harmful for neurons. In contrast, A2 is an anti-inflammatory state and provides protection for the neurons. Depending on the trigger, astrocytes, when activated, will transform to either one of the two phenotypes. During normal ageing, despite any injury or disease, astrocytes will become reactive. Gene expression measured in aged astrocytes showed that the astrocytes take on the pro-inflammatory phenotype A1, especially in the striatal and hippocampal area [24]. This new phenotype has shown to induce neuronal cell death and prevention of it proved to be neuroprotective [25].

2.1.1 Microglia

Microglia cover 10% of all glial cells in the CNS and were first believed to function solely as a defence mechanism against injury [22, 26]. However, new findings suggest their involvement in other processes as well, such as synaptic remodelling and the survival of cortical neurons [27, 28]. When they do not contribute to these processes, microglia are still metabolically active. They are always scanning their environment searching for injury or other abnormalities and will respond to such a trigger by starting to migrate to the site of injury and by the transformation of their morphology and function [29, 30]. This is termed microgliosis and comes with many changes including the secretion of pro-inflammatory mediators. These mediators are meant to prevent further infection but can be toxic to their environment as well [22]. Additionally, microglia will produce and release toxic oxygen- and nitrogen-derived products, such as superoxide and nitric oxide, which are toxic for their surrounding cells [31].

More microglia seem to take on this active phenotype during ageing and this could lead to the development of an inflammatory environment. Indeed, an increase in the production of pro-inflammatory cytokines

in astrocytes is seen during ageing and microglia showed increased expression of TNF-α, IL-1β, IL-6 and IL-10 when they were repeatedly activated with LPS [32, 33]. Furthermore, Chao et al. (1992) showed that microglia are capable of inducing neurotoxic effects in a NO-dependent manner after being exposed to LPS and IFN-γ. Activated microglia are capable of activating astrocytes as well. IL-1α, TNF and C1q, secreted by microglia, transformed astrocytes to their A1 phenotype and they lost the ability to promote neuronal survival, outgrowth and synaptogenesis and they induced neuronal death [25].

2.2 Inflammation in Parkinson's Disease

In the last two decades, a large amount of evidence is gathered supporting the pro-inflammatory environment in PD. In PD patients, levels of pro-inflammatory mediators such as IL-1β, IL-6, epidermal growth factor (EGF) and TNF-α are significantly elevated in the CSF and the dopaminergic and striatal regions of the brain [35, 36, 37]. Moreover, the receptor for TNF-α is upregulated in dopaminergic neurons in the SN, making them more susceptible for this pro-inflammatory cytokine, and therapy with anti-inflammatory drugs seems to decrease the risk on PD [38].

It was McGeer et al. in 1988 who for the first time proved the involvement of microglia in PD. They found, after analysing post-mortem PD brains, an increase in activation of microglia in the SN. Following research confirmed these findings and showed an increased production of TNF-α, IL-1β, IL6 and NO in the activated microglia [40, 41, 42]. In addition, several toxins, who mimic symptoms of PD, act through the activation of microglia. Neuromelanin, after injection in SN, caused degeneration of the dopaminergic neurons and was found to be releasing NO, IL-6 and TNF-α from the microglia [43]. 1-methyl-4-fenyl-1,2,3,6-tetrahydropyridine (MPTP), a drug that is used as a model for studying PD because of its neurodegeneration in dopaminergic regions of the SN, also acts through the activation of microglia [44]. Lastly, the orphan nuclear receptor Nurr1 prevents the expression or pro-inflammatory mediators in astrocytes and microglia and, therefore, protects against the loss of dopaminergic neurons in the SN [45].

In contrast to microglia, the phenotypical changes in astrocytes are less severe. However, there is enough evidence to support an inflammatory reaction of astrocytes in PD. In a study where α-synuclein was overexpressed in mice, Gu et al. (2010) found evidence of astrogliosis and saw that this activation led to an inflammatory response, the activation of microglia and neurodegeneration. In addition, another study found increased activation of microglia and astrocytes in the SNpc of chronic Parkinsonian macaques [47]. The expression of pro-inflammatory mediators in activated astrocytes is increased as well. The activated astrocytes in PD increase their secretion of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α [48, 49]. Lee et al. (2010) surprisingly showed that α-synuclein is capable of aggregating in astrocytes which causes the production of IL-1α, IL-1β and IL-6 and the upregulation of the genes for TNF-α, IL-1 and IL-6. Furthermore, this secretion has shown to contribute to the neurodegeneration in the SN [49, 51].

3 Cellular senescence

Cellular senescence is a state of permanent cell arrest and can be induced by telomere shortening [55, 56, 57], DNA damage [58], ROS [59], oncogenic stressors [60, 61, 62], chromatin disruptions [63] and by SASP components of other senescent cells [64]. These triggers all create a different phenotype which makes identification of senescent cells a complex process. Besides the detrimental effects of senescent cells on the environment, cellular senescence also supports beneficial biological processes such as wound healing and tumour suppression [65]. Currently, multiple markers are simultaneously used to identify senescent cells, all based on the typical changes seen in senescent cells and the signalling molecules from general molecular pathways underlying the cell-cycle arrest or the SASP. Common markers used to identify senescent cells include p16^{INK4a} (hereafter p16), p53, the senescence associated β-galactosidase (SA-βgal), a flattened and enlarged cell morphology, decreased lamin B1 expression and senescence associated heterochromatin foci (SAHF) [66]. Box 1 shows an overview of the main characteristics of senescent cells. However, because of the large diversity in senescence, not every characteristic is seen in a senescent cell.

It is hard to provide a general composition and size of the SASP as every trigger and every different type of cell creates a different senescent phenotype. However, of all the cytokines expressed in the SASPs, IL-6 and IL-8 seem to be the most abundant [67]. In general, the SASP can be subdivided into 3 categories: soluble factors, secreted proteases, and secreted insoluble proteins/extracellular matrix components [5]. The size of the categories depends on the trigger and type of cell, however the soluble factors do seem to cover most components [5]. Shelton et al. (1999) were the first to identify the SASP by microarray analysis on dermal fibroblasts, retinal pigment epithelial cells and vascular endothelial cells after repeated replication.

BOX 1	Overview of the main characteristics of senescent cells

- **Cell cycle arrest.** The most popular characteristic of senescent cells is the stop on the cell proliferation. Hayflick and Moorhead were the first to identify this arrest [1,2]. This arrest is irreversible *in vivo* but can be reversed *in vitro* by targeting specific signalling molecules [52].

- **Flattened and enlarged morphology.** During senescence a cell starts to take on a flattened and enlarged morphology. This change has been linked to the cytoskeletal protein Vimentin, mTOR activation and ·ATF6α signalling [66].

- **Senescence associated secretory phenotype (SASP).** The SASP is a cell's new secretome after becoming senescent. The composition of the SASP differs but is best known for its pro-inflammatory component and its capability to affect the micro-environment of a cell [5].

- **Senescence associated heterochromatin foci (SAHF).** Many changes in the expression of genes in senescent cells are a consequence of newly formed heterochromatin foci. These foci suppress the transcription of genes related to the secretome of a cell and the cell-cycle progression [123]. The formation of SAHFs is mainly seen in senescence induced by oncogenes [66].

- **Senescence associated β-galactosidase (SA-βgal).** Almost all senescent cells show activity of the enzyme β-galactosidase at pH = 6.0, a phenomenon termed SA-βgal [53]. In non-senescent cells, lysosomal β-galactosidase is active around pH = 4.5 making the measurement of activity around pH = 6.0 a distinctive marker for senescence. The origin of the upregulation of β-galactosidase activity at pH = 6 is unknown but it is one of the first and most common used marker for cellular senescence [6, 66].

- **Loss of Lamin B1 expression.** Laminas are proteins important for the structure of the nucleus. In senescent cells the expression of lamin B1 is decreased, causing a change in the structure and size of the nucleus [54, 66]. Additionally, this reorganisation is capable of affecting the gene expression as well [66].

- **Upregulation of signalling molecules from the DNA damage response (DDR).** The DDR is the main pathway behind senescence and the upregulation of DDR components are therefore used as markers for identifying senescence. An increase in molecules such as $P16^{INK4a}$, $p21^{Cip1}$ and p53 are commonly used as markers for senescence [66].

They found a clear increase in the mRNA expression of proteins involved in inflammatory processes and following research showed the presence of many other molecules including cytokines, chemokines, growth factors, and proteases, most of them part of the soluble factors [5, 67, 69]. All of these factors are capable of influencing biological processes in a beneficial or deleterious way and the SASP is considered to be the most influential component of the senescent phenotype [70].

3.1 DNA damage response

While there are many different triggers capable of inducing senescence, most of them act through the DNA damage response (DDR). The shortening of telomeres is the best known trigger for senescence and it has been proposed that it activates the DDR by two processes. First, when a telomere is too short, it is unable to form a T-loop causing the activation of ATM and ATR, two downstream signalling molecules of the DDR [72, 73]. Second, when a telomere shrinks, the telomeric binding proteins Telomeric repeat-binding factor 2 (TRF2) and Protection of telomeres (POT1) decrease in concentration [71]. These proteins are normally bound to the telomeres and inhibit the activation of ATM and ATR. However, when the telomeres become shorter, less TRF2 and POT1 will be bound, causing the activation of the DDR [71].

Oncogenic stressors also act through the activation of the DDR. These stressors either cause a hyper-replication phase, in which there is a high rate of mistakes in the replication of the DNA [61, 74], or will activate $p19^{ARF}$ from the DDR [7]. Double strand breaks (DSBs) and single strand DNAs (ssDNAs) arise from the mistakes made in (hyper-)replication and will initiate the onset of the DDR. In addition, DSBs and ssDNAs also arise when damage is done to the DNA by, for example, oxidative stress. Other stressors such as chromatin disruptions and SASP molecules act either directly on the pathways described below or will change the expression or chromatin formation of DNA to either upregulate genes related to senescence or to downregulate genes related to cell-cycle progression [63, 64]. Figure 1 shows a overview of the main triggers of the DDR.

DSBs are recognized by the DNA repair complex MRN consisting of the nuclease Mre11, and the two proteins Rad50 and Nbs1 [75]. Besides the MRN complex, additional factors are recruited to the site of DNA damage including BRCA1 [76], p53-binding protein 53BP1 [77] and mediator of DNA-damage checkpoint protein 1 (MDC1) (figure 1) [78]. After binding, these factors will activate ataxia telangiectasia mutated (ATM). ssDNAs are recognized by an interaction of Tipin and the replication protein A (RPA) who together cause the activation of ataxia telangiectasia and RAD-3 related (ATR) (figure 1 & 2) [79, 80]. ATR is especially activated by nucleotide depletion and when the replication of the DNA is blocked [81].
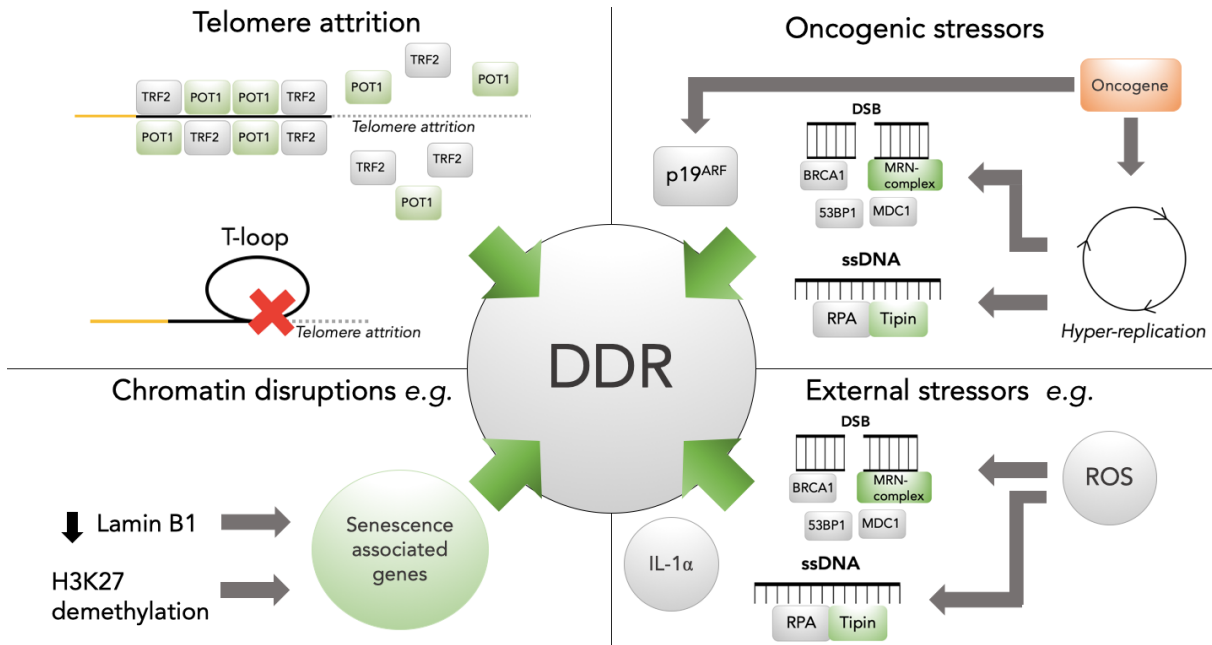
Figure 1: **Overview main triggers of DDR.** Telomere attrition causes the activation of the DDR when the telomere is unable to form a T-loop or by loss of the telomere binding proteins: Telomeric repeat-binding factor 2 (TRF2) and Protection of telomeres (POT1) [71]. Oncogenic stressors activate either p19^{ARF} or cause a hyper-replication phase which produces DSBs and ssDNA [61]. Chromatin disruptions affect the transcription of senescence associated genes and are in this way capable of activating the DDR as well [63]. Additional external stressors are capable of producing DSBs or ssDNA or will act directly on DDR components [64]. There are many more ways to induce the DDR by chromatin disruption and external stressors than those given in this figure.

3.1.1 Regulation cell cycle arrest

The first reaction of the DDR is to phosphorylate the histone H2AX to provide chromatin changes necessary for the recovery of the DNA [86, 87]. The second reaction is to stop the cell-cycle, this is the first and most prominent phenotypical change in the senescent cells. ATM and ATR initiate the arrest by phosphorylating checkpoint kinase (CHK) 1, CHK2 and tumor protein p53 [88].

Once CHK1 is activated, it will act on its substrates important for the cell-cycle control such as CDC25s [88]. Activated CHK2 initiates many cell-cycle related processes including the phosphorylation of p53 [81, 89]. P53 will continue with downstream signaling to eventually initiate the senescent phenotype. Besides CHK2 and ATM/ATR from the DDR, the oncogene/p19^{ARF} pathway is also capable of phosphorylating p53 [7, 90]. p19^{ARF} is mainly activated by stressors such as oncogenes but DSBs are capable of doing this as well [91, 92]. When activated, p19^{ARF} is capable of stabilizing and inhibiting the human double minute 2 homolog (HDM2) [93]. Normally, HDM2 is responsible for the degradation and the inhibition of p53 [94, 95]. However, when HDM2 is inhibited, more p53 will be activated and available. Subsequently, p53 is capable of binding to the promoter of the cyclin-dependent kinase inhibitor 1 p21^{Cip1} (hereafter p21) [83, 96].

P21, the cyclin-dependent kinase inhibitor p16^{INK4a} (hereafter p16) and CDC25s alter the activity of cyclin-dependent kinases (CDKs). These kinases are important for the progression of the cell-cycle and inhibition of them will stop cell proliferation [97]. P21 is capable of inhibiting the CDKs 2, 3, 4 and 6 but seems to prefer inhibiting CDK2 [98, 99]. CDC25, normally activates CDKs, however, because of the inhibition of CDC25 by p53, this is prevented [79]. The last activator, p16, is activated by several triggers like oncogenes and DNA damage but in a more complex manner involving epigenetic control and transcription factors. One important activation mechanism is by the inhibition of Polycomb group repressor complexes [100, 101]. In general, p16 is activated by the inactivation of its suppressors [102]. Unlike p21, p16 does not bind to CDK2 & 3 but only binds to the CDKs 4 & 6 [103]. Additionally, the activation of p16 seems to appear later in the onset of senescence probably through a regulatory mechanism involving the demethylation of H3K27 [103, 104].
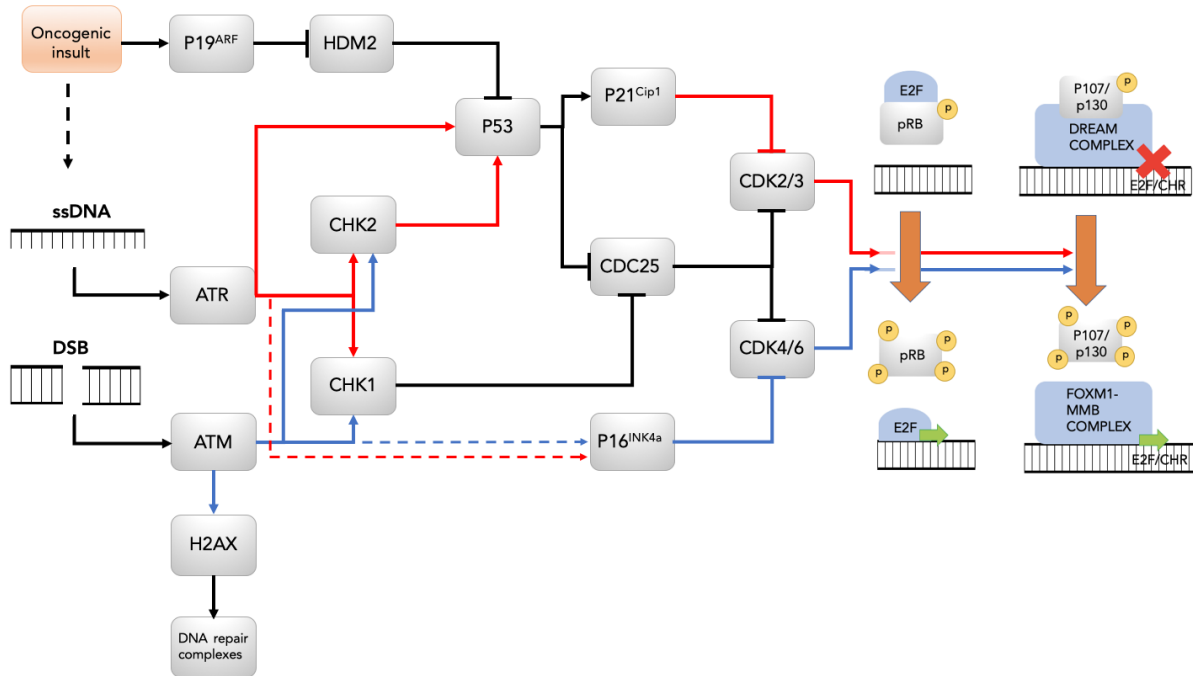
Figure 2: **Molecular Pathway - from DNA damage response to cell-cycle arrest.** Most triggers causing senescence can be traced back to the activation of the DDR. The DDR is initiated by a response to a single strand DNA (ssDNA) or a double strand break (DSB). After this, ATR or ATM is activated causing the activation of different pathways. Oncogenic stressors cause ssDNA and DSB as well, by inducing hyper-replication, but are also capable of activating p19[ARF] [7]. After this, a cascade of reactions occur, all eventually leading to the inhibition of the hyperphosphorylation of pRB, p107 and p130 [82, 83]. The hypophosphorylated state of pRB prevents E2F transcription factors to start the transcription of cell-cycle related genes [84]. p107 and p130, in their hypophosphorylated form, activate the DREAM complex which will prevent transcription factors to bind to the promoter of E2F and CHR elements, both important for the progression of the cell cycle [82]. In addition, activation of the DREAM complex also leads to modifications in the chromatin structure [85]. The lines towards p16[INK4a] are dotted to emphasize the underlying indirect complex activation which is not included in this figure. Colours are used to provide contrast, not to highlight different functions or processes.

The inhibition of the CDKs prevents the hyperphosphorylation of the retino blastoma proteins (pRB), retinoblastoma-like protein 1 (p107) and retinoblastoma-like protein 2 (p130) [82, 83]. The hypo-phosphorylated pRB will bind to E2F and prevents its binding to the promoter of cell-cycle promoting genes. P107 and p130, in their hypo-phosphorylated state, bind to the FOXM1-MMB complex to form the DREAM complex [83, 84, 96]. This complex is capable of binding to the promoter of E2F and CHR elements in the DNA and prevents expression of genes important for the cell proliferation by the blocking of the promoter and the formation of heterochromatin structures [96, 105, 106]. This is one of the pathways responsible for the formation of the SAHFs [85]. Figure 2 provides a summary of the initiation of cell-cycle arrest by the DDR.

3.1.2 Regulation of the senescence associated secretory phenotype (SASP)

The regulation of the cell-cycle arrest is much better understood than the pathways underlying the SASP. Most evidence points towards two major transcription regulators responsible for the expression of the SASP: the CCAT/enhancer binding protein β (C/EBPβ) and NF-κB [107].

NF-κB regulates the expression of many, but not all, pro-inflammatory genes of the SASP [108, 109]. The DDR is capable of regulating NF-κB mainly by ATM. Silencing of ATR only showed a small decrease in the expression of the SASP whereas the effect of ATM was large [110]. ATM has been proposed to regulate NF-κB in different ways. For example, ATM is capable of phosphorylating the protein NEMO, a regulatory subunit of the IKK complex. As a results IKKα/β will inhibit the inhibiting subunit of NF-κB called IkB [111]. ATM also showed to be an activator of p38 mitogen-activated protein kinases (P38MAPK) [112, 113]. P38MAPK has shown to activate NF-κB by causing p300, a coactivator of NF-κB, to acetylate the subunit

p65 of NF-$\varkappa$B [108, 114]. In addition, P38MAPK was able to activate p16, p53 and pRB, and was directly activated by telomere shortening and other cellular stressors [115]. Thus, p38MAPK seems to be more than only the activator of the SASP in senescent cells.

The DDR can't be the only regulator behind the development of the SASP. While the cell-cycle arrest occurs directly after the onset of senescence, the SASP only reveals itself after a couple of days [67]. Therefore, other pathways have to be involved. IL-1α is a mediator which appears to be important. IL-1α is part of the SASP and is capable of activating NF-$\varkappa$B by acting on IL-6 and IL-8 [116]. Furthermore, IL-6 and IL-8 are part of the SASP creating a positive feedback loop important for maintaining the expression of the SASP [5, 67, 69]. IL-1α creates another positive feedback loop because its expression is elevated by mTOR [117]. In many senescent cells the levels of mitochondria are increased as a result of the senescent phenotype [118], causing an increase in the level of mTOR [119]. mTOR acts through IL-1α to induce senescence and therefore increases the number of mitochondria as well. This positive feedback loop may play a part in maintaining the senescent phenotype as well.

IL-1α, IL-6 and IL-8 also influence the activity of C/EBPβ [69, 116]. C/EBPβ is part of the basis leucine zipper superfamily where only the β isoform is capable of inducing senescence [107]. Overexpression of C/EBPβ is sufficient to induce senescence and it is very likely doing this by its influence on the IL-6 and IL-8 expression [69]. Especially oncogenes seem to influence the SASP by C/EBPβ. Several studies have shown a strong increase of C/EBPβ when senescence was induced by oncogenes [120, 121].

3.2 Chromatin modification

Chromatin modification also play an important part in the onset of senescence and the SASP. Shah et al. (2013) showed that H3K27 demethylation affects 65.1% of the SASP related genes in IMR90 cells, a cell line from the female foetal lung. Genes for Matrix metallopeptidases (MMPs), who are part of the SASP and capable of degrading extracellular matrix proteins, were shown to be the most affected but other molecules from the SASP were influenced as well [122]. They also showed that the lamin B1 down expression, often seen in senescent cells, is probably the reason behind this demethylation and causes chromatin changes which allows the transcription of the SASP genes. Aird et al. (2016) showed another mediator for chromatin modification, high mobility group box 2 (HMGB2). HMGB2 regulates the expression of SASP genes such as MMPs, chemokines and cytokines and is doing this by preventing SAHFs to spread into SASP gene loci. During senescence, HMGB2 is upregulated and loss of the protein blunts the expression of SASP genes. However, the cell-cycle arrest maintains indicating a specific role for this protein in the regulation of the SASP [123]. In addition, the mediators of the DDR are capable of altering the chromatin structure as well by activating chromatin-modifying proteins and by preventing the hyperphosphorylation of pRB [124, 125].

4 Senescence in astrocytes

It was Evans et al. (2003) who for the first time really showed proof of senescence in astrocytes. Their cultured primary human astrocytes stopped proliferating and showed elevated levels of p21 and SA-βgal after 20 passages. They investigated whether the DDR was responsible for the initiation of senescence and found that after abrogation of p53 and p16, the life-span increased, indicating an important role for both mediators in inducing senescence [126]. Pertusa et al. (2007) showed that after 90 days, their cortical astrocytes isolated from rats started to stain positive for SA-βgal and, more importantly, these astrocytes showed an inability to maintain neuronal survival. They found an upregulation of glutamate uptake, decreased mitochondrial activity, the production of ROS and elevated iNOS levels in the astrocytes [127]. Other research showed that senescence is initiated in astrocytes by extracellular stressors as well. In a study looking at the effect of H_2O_2 and the proteasome inhibitor lactacytstin-2 on astrocytes, they discovered that 90% of all the exposed cells showed an enlarged morphology and increased expression of SA-βgal. Furthermore, these changes were accompanied by elevated expression of p21, p16, p53 and SAHF [128].

Research concerning astrocyte senescence only just started to look at their secreted phenotype but is already showing evidence of its contribution to inflammation. The secretory phenotype of astrocytes from Wistar rats was examined by exposing them to H_2O_2 and proteasome inhibitors *in vitro*. They found that mainly the chemokines GROα, IP-10, MCP-1α, MIP-2 and RANTES were secreted by the senescent astrocytes. These chemokines are capable of inducing the clearence of cells by activating the immune system [129]. Surprisingly, they found little expression of IL-6 and IL-8 compared to the SASP of fibroblasts. On the contrary, the cytokine IL-1α, which is capable of activating IL-6 and IL-8, showed a higher expression [129]. Crowe et al. (2016) investigated changes in the transcriptome in, by oxidative stress induced, senescent
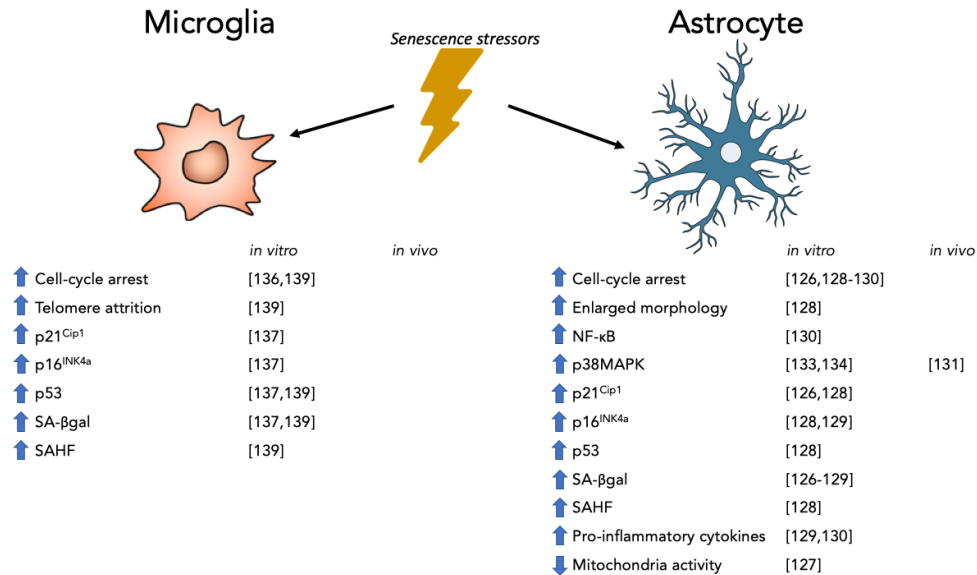
Figure 3: **The current evidence for senescence in astrocytes and microglia.** Different senescence stressors are capable of inducing senescence. Most evidence comes from cultured cells where cells are exposed to stressors such as repeated replication, H_2O_2 and proteasome inhibitors.

astrocytes. Pro-inflammatory genes were found to be upregulated, including NF-$\varkappa$B, while genes involved in neuronal development and differentiation were downregulated [130]. These studies indicate that when an astrocyte becomes senescent, its phenotype becomes inflammatory. Indeed, the pro-inflammatory genes who are expressed in active A1 aged-astrocytes show overlap with the genes expressed in the SASP of senescent astrocytes [24].

P38MAPK seems to play an important role in initiating astrocyte senescence. In human post-mortem Alzheimer's Disease brains the senescent astrocytes showed a strong upregulation of P38MAPK [131]. Ginsenoside F1, an inhibitor of P38MAPK and NF-$\varkappa$B, caused the inhibition of the SASP, indicating an important role for NF-$\varkappa$B as well [132]. Two other studies where ammonia was used to induce senescence revealed a mechanism involving P38MAPK [133, 134]. In addition, one of them showed a depletion of senescence when P38MAPK was inhibited [133]. Lastly, a logical model created of the most prominent pathways known behind the SASP showed P38MAPK as the most important mediator in the onset of the SASP [135].

5 Senescence in microglia

Current research on microglia senescence is far less extensive than the research focused on astrocyte senescence. In their culture experiments, Flanary & Streit (2004) were the first to describe senescence in microglia. After analyzing the telomeres in microglia and astrocytes, they found telomere shortening in microglia but, surprisingly, not in astrocytes [136]. In contrast to the findings of Flanary & Streit, Stojiljkovic et al. (2019) found no telomere shortening or other sign for senescence in the microglia of murine aged brains (24 months). The only senescent marker increased in the microglia was p16 [137]. The low turnover rate of microglia relative to the short life span of mice may be responsible for this discrepancy [138]. The *in vitro* experiments performed by Stojiljkovic et al. (2019) showed, however, clear indications of senescence in microglia. In the later passages of the experiments, the microglia cells showed a 9-fold increase of the expression of SA-βgal, chemokines related to inflammation and mediators of the p53/p21 pathway [137]. In a study where BV2 microglia were repeatedly activated by LPS, cell-cycle arrest was induced at the G1 phase, typical for cellular senescence [74, 139]. Additionally, the levels of the senescent markers p53, SA-βgal and SAHF were elevated in the microglia [139]. In order to understand the role of senescence in the CNS, more research is required on microglia senescence. The current evidence on senescence in microglia is minimal and not sufficient to conclude their presence in the aged brain. However, their influence on neurodegenerative diseases may be critical because of their normal functions and their potential to create an inflammatory environment [138].

6 Astrocyte and microglia senescence in PD

Because of the detrimental effects of the senescent cells to their environment, senescence has been linked to numerous pathologies, including age-related neurodegenerative diseases. One of the ideas is that the pro-inflammatory component of the SASP enhances the chronic neuroinflammation, which is believed to be deleterious in these disorders. Moreover, senescent cells accumulate over time making the effect of senescent cells more profound in age-related disorders [6]. Indeed, astrocyte and microglia senescence has already been identified in, respectively, Alzheimer's disease and Amyotrophic lateral sclerosis and removal of senescent cells attenuated the symptoms in both [131, 140]. These findings suggest a role for astrocyte and microglia senescence in PD as well and research into this may lead to novel therapeutic options for PD.

The first signs of senescence in PD came from the identification of elevated levels of pRB in the SN [141] and SA-βgal in the CSF [142]. However, in contrast to these findings, the meta-analysis performed by Forere et al. (2016) showed no signs of telomere attrition in post-mortem PD brains, indicating the absence of senescence. This does not have to imply anything as the onset of senescence is not triggered by the average telomere length but by the presence of only a few shortened telomeres [73, 144]. Furthermore, as shown before, senescence can be induced by numerous triggers and is not solely dependent on the shortening of telomeres.

Additional evidence for senescence in PD came from studies exploring the mechanism behind external triggers causing PD, such as 2,3,7,8 – tetrachlorodibenzodioxin (TCDD). TCDD is an environmental contaminant which is believed to be a cause for PD [145]. Nie et al. (2015) investigated the mechanism behind this toxin and identified astrocyte senescence as a possible target. Astrocytes exposed to TCDD showed elevated markers of SA-βgal, p16 and p21 [146]. In addition, they discovered cytoskeletal remodelling in the cells and identified WNT/β-catenin and ROS signalling as underlying regulators [146]. Lee et al. (2010) investigated the role of senescence by inhibiting glutathione (GSH) biosynthesis with d,l-buthionin-S,R-sufloximine (BSO). The inhibition of GSH causes oxidative stress which is known as a contributor to the pathogenesis of PD [147]. As a response to the inhibition, they found astrocytes and microglia who were triggered to release TNF-α, IL-6 and nitrite ions. Moreover, the release of these molecules was related to the activation of P38MAPK and NF-ϰB [50]. Although they did not use the conventional markers for senescence, the presence of P38MAPK and NF-ϰB activation points towards a role of senescence in this process.

The first and only study to really focus on the role of senescence in PD *in vivo* was performed by Chinta et al. (2018). They provided the first direct evidence of astrocyte senescence in PD. In the analysis of the SNpc from post-mortem PD brains, they discovered elevated levels of p16, IL-6, IL-1α, IL-8 and MMP and decreased levels of lamin B1, all indicators of senescence [148]. In addition, they looked at the effect of Paraquat (PQ) on astrocytes, a neurotoxin associated with an increased risk on PD. They discovered a stop on astrocyte proliferation after astrocytes were exposed PQ *in vitro* and found increased levels of p16, IL-6, SA-βgal and 53BP1 [148]. The astrocytes who stopped proliferating also reduced the viability of the neurons and suppressed the NPC proliferation and migration. Lastly, PQ was given to p16-3MR mice, an animal model capable of selective depletion of senescent cells [149]. After the removal of the senescent cells the neurogenesis went up, the number of dopaminergic neurons increased and the locomotor function of the mice was improved [148]. Together, these data clearly indicate a detrimental effect of astrocyte senescence in the PD pathology and create the suggestion for a possible new therapeutic intervention for PD by targeting the senescent cells.

7 Conclusions & future perspectives

The current evidence on astrocyte and microglia senescence in PD is scarce but shows promising results. *In vitro* research on senescence in astrocytes and microglia shows that both cell types are capable of becoming senescent, however, the extent of their prevalence *in vivo* is still largely undiscovered. The conclusions drawn from senescent research *in vitro* are hard to translate to *in vivo* as astrocytes and microglia are subjected to multiple stressors simultaneously, in contrast to cultured cells who are only subjected to one or two. Therefore, more *in vivo* evidence is definitely needed to confirm astrocyte and microglia senescence as a component of ageing and disease.

Currently, most research on senescence in glial cells is performed on astrocytes as it is believed that this glial cell will present the most profound effect of senescence because of its many homeostatic functions. However, microglia, as stated before, play several homeostatic functions as well and are related to several pathologies, including PD, making their senescence also of importance. More research is definitely required on both to

discover their molecular mechanism behind senescence and to further examine their presence and influence. When we understand the mechanisms underlying the senescent phenotype in these cells, we could possibly target molecules from the pathways to reduce the pro-inflammatory component or other detrimental effects of senescence. Additionally, an interesting notion proposed by Cohen and Torres is that many studies which examined the role of reactive astrocytes in biological processes may actually have been looking at senescent astrocytes instead [150]. The similarities between the phenotypes of reactive and senescent astrocytes, such as the enlarged morphology and the secreted pro-inflammatory mediators, create the possibility that without clear identification, the studies actually looked at senescence [24, 128]. Therefore, further elucidation of their prevalence may be of importance for a better understanding of biological processes.

Whether the senescence of astrocytes and microglia plays a role in PD needs to be further investigated but for now their contribution, especially from senescent astrocytes, seems to be there. The PD-inducing toxins, contributing to the initiation of senescence in astrocytes and microglia, and the presence of elevated senescent markers in PD patients shows evidence of a role of astrocyte and microglia senescence in PD. In addition, senescent astrocytes and microglia investigated without the presence of a pathology show their capability of producing pro-inflammatory molecules and could therefore contribute to the neuroinflammation in PD. Chinta et al. (2018) were the first to produce *in vivo* evidence of astrocyte senescence in PD and, more importantly, gave proof for a contribution of senescence to the pathology. It would be interesting to further discover why exactly the senescent cells were detrimental in this model and whether senescent microglia occur in PD as well.

Future research should aim to further clarify the specific role of astrocyte and microglia senescence in PD. PQ is one of the many models to study PD and is not a perfect mimic of the disease [151]. To really confirm the influence of astrocyte and microglia senescence in PD, other PD models have to be applied to senescent animal models such as INK-ATTAC and p16-3MR. These transgenic models are capable of removing senescent cells by targeting p16 promoting cells and are, therefore, very useful in studying the role of senescence [149, 152]. However, p16 expression is not limited to senescent cells and not all senescent cells promote p16 [137, 153]. In addition, they don't provide specificity for types of cells. Novel methods capable of removing specific senescent cell types would allow to identify which senescent types contribute to the pathology, and to what extent. This discrimination between cell types could benefit the development of senescence removal drugs (senolytics) as well, as they currently also remove the beneficial effects of senescent cells such as tumour suppression.

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