The Role of Senescence in Cardiovascular and Neurodegenerative Diseases

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Abstract

The population of the world is increasing in lifespan and more and more people are aging, with aging the risk of developing various diseases increases. In aging, there is a higher chance of developing cardiovascular and neurodegenerative diseases.

Senescence, a steady cell cycle arrest, is thought to be a hallmark of aging and there are connections between the onset of age-related diseases and senescence. However, the exact role of senescence in cardiovascular and neurodegenerative diseases is not fully understood yet. In this thesis, the role of senescence in cardiovascular and neurodegenerative diseases is investigated.

Senescent human vascular endothelial cells and human vascular smooth muscle cells excrete proinflammatory- and other factors resulting in vascular calcification and the onset of atherosclerosis. Atherosclerosis is a cardiovascular disease itself but can result in the development of more serious cardiovascular diseases, such as coronary heart disease. Senescent microglia and astrocytes cells increase neuroinflammation and formation of neurofibrillary tangles, resulting in the onset of Alzheimer's and Parkinson's disease which are the two most common neurodegenerative diseases.

The results shown in this thesis show that there is a connection between the onset of senescence and the onset of cardiovascular and neurodegenerative diseases. This connection is in the form of four different cells; senescent human vascular endothelial cells, senescent human vascular smooth muscle cells, senescent microglia and senescent astrocytes. These senescent cells increase the risk of developing cardiovascular and neurodegenerative diseases. Following these findings, it is anticipated that future research on the role of senescence in these diseases will be done. For example, research into specific compounds targeting senescent microglia to improve the onset of neurodegenerative diseases.

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Introduction

The average lifespan of humans is increasing, at this moment 12% of the human population is 65 years or older. This percentage is expected to increase to 22% of elderly people in 2050 (*Ageing and Health*, 2021). This seems like a good development, but aging is a risk factor for the onset of different diseases such as cardiovascular diseases, neurodegenerative diseases, diabetes and cancer (Jaul & Barron, 2017). Aging is defined as the progressive loss of organ and tissue function over time according to Flatt (2012). Senescence is associated with aging and is even seen as a hallmark of aging (López-Otín et al., 2013). Senescence is a condition in which cells stop proliferating and stay in a stable cell cycle arrest. This is beneficial if for example a tumor cell becomes senescent and stops proliferating. But not every senescent cell has a beneficial effect on the body (Coppé et al., 2008). This is due to the development of the so-called Senescence-Associated Secretory Phenotype (SASP) which is a phenotype of senescent cells resulting in the excretion of different types of factors into the environment around the senescent cell. This can lead to inflammation of the environment but can also lead to e.g. tumor proliferation.

The group of diseases that kills the most people worldwide are cardiovascular diseases (CVDs), which take around 17.9 million lives every year (*Cardiovascular Diseases*, 2019). Furthermore, the medical bill was found to be $10,345 higher for people suffering from CVDs, and in low-income countries the total medical bill rose to 3.35 trillion dollars (Foy & Mandrola, 2018). The increase in the lifespan in humans is associated with an increased risk for the development of CVDs.

Next to CVDs, neurodegenerative diseases are a group of diseases that also affect people worldwide. With the onset of a neurodegenerative disease different costs come into play, such as the costs to put a patient in hospital but also costs of medicines and rehabilitation. According to Sopina et al. (2018), the medical bill of a person with Alzheimer's Disease (AD) was €4996 higher a year than the 'healthy' control group. With around 6.2 million suffering from AD last year in the United States only, the economic burden of neurodegenerative diseases is huge and while the deaths from CVDs are decreasing since 2000, the deaths from AD have increased by 145% since 2000 (Alzheimer's Disease Facts and Figures, 2021).

The increase in age is accompanied by an increase in senescent cells and this might be interconnected with each other (Mylonas & O'Loghlen, 2022). An increase in senescent cells could be the cause of the increased chance of developing cardiovascular and neurodegenerative diseases. While there are different cells in the environments of cardiovascular and neurodegenerative diseases. All senescent cells, in general, share characteristics of which the SASP is the most important one.

Because cardiovascular and neurodegenerative diseases have had and still have a huge impact on society this thesis will look into the role of senescence in the development of these diseases. Thus, the research question of this thesis is '*What is the role of senescent cells in the development of Cardiovascular and Neurodegenerative diseases?*' To tackle this research question the aim is to first provide some information about senescence and the two diseases themselves, to then go into depth and see what the connection between the two diseases and senescence is.

Chapter 1: Cellular Senescence

Cellular Senescence

Cellular senescence is a state in which the cell cycle becomes arrested and proliferation stops (Kumari & Jat, 2021). Cells arrest in the G1/S phase of the cell cycle. The onset of senescence in cells is triggered by the activation of one of two tumor suppressor networks. These two networks are the $p16^{INK4A}$/Rb and the $p53/p21^{CIP1}$ networks. The $p53/p21^{CIP1}$ network can be activated in response to DNA damage and is thought to be an initiator of senescence. The response to DNA damage is called the DNA Damage Response (DDR), DDR activates different stress sensors such as telangiectasia-muted (ATM) and Rad3-related (ATR) kinases. These kinases activate the $p53/p21^{CIP1}$ networks by phosphorylating p53 via Chk1/2 resulting in the activation of P21, which inhibits the cyclin- CDK complex (see figure 1)(Mijit et al., 2020). The $p16^{INK4A}$/Rb network is connected to multiple proteins of the Rb family, $p16^{INK4A}$ binds to CDK4/6 and prevents the formation of cyclin-CDK complexes. This results in the blockage of Rb phosphorylation, which prevents promoting the expression of E2F genes which are necessary for the cell cycle transition (see figure 1) (Kumari & Jat, 2021). The $p16^{INK4A}$/Rb network seems to have a role in maintaining senescence, as loss of the $p16^{INK4A}$/Rb network leads to the promotion of cancer (Pérez-Sayáns et al., 2015). Ultimately, both these two tumor suppressor networks act on the cyclin- CDK complex which phosphorylates retinoblastoma (Rb) causing expression of E2F genes which result in cell transition from the G1 to the S phase. Inhibiting this complex results in a stable cell cycle arrest (see figure 1) (McHugh & Gil, 2017).

Different intrinsic and extrinsic signals can trigger $p16^{INK4A}$/Rb and the $p53/p21^{CIP1}$ networks (see figure 1). As mentioned before, DNA damage is a trigger for senescence because it activates a DDR. Endogenous DNA damage is researched and verified as a driver of senescence (Niedernhofer, 2019). Next to this telomere shortening is a trigger for senescence (see figure 1)(Fagagna et al., 2003). Dysfunction of the autophagy pathway can also cause the onset of senescence (Moreno-Blas et al., 2019). Next to these pathways, also some genes are associated with triggering senescence. Three genes are associated with the accumulation of senescence. PRODH, DAO and EPN3 were all found to trigger senescence all three genes are regulated by p53 which, as mentioned before, is shown to have an essential role in senescence induction (Nagano et al., 2016). Overexpression of these three genes leads to senescence and a byproduct of the overexpression of these genes is ROS production, which can lead to DNA damage and thus senescence (Nagano et al., 2016).

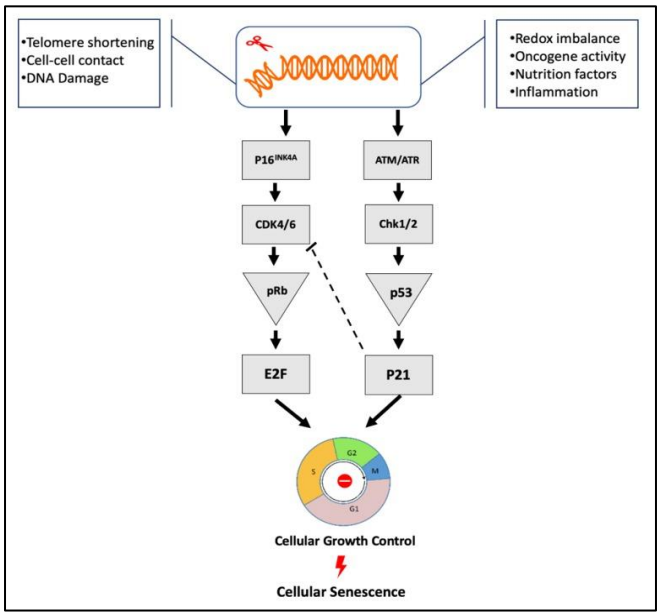

Figure 1. The two tumor suppressor networks P16INK4A/Rb and p53/p21CIP1. When activated, the p16INK4A/Rb and the p53/p21CIP1 networks will lead to the onset of senescence by affecting downstream components and ultimately inhibiting CDK4/6 (Mijit et al., 2020).

Senescence and Cancer

Cellular senescence is thought to be an anti-cancer mechanism, tumor cells that become senescent cannot proliferate and invade the body anymore. Senescent cells can influence the immune system and thereby limit cancer development (Kang et al., 2011). Next to having a detrimental effect on cancer development, unfortunately, senescent cells can also have a beneficial effect on cancer development. Research has shown that senescent cells excrete different factors during the development of the SASP. The excretion of all these different factors during the SASP can promote tumor proliferation (Coppé et al., 2006) (Ouchi et al., 2016). There is also oncogene-induced senescence (OIS), in which due to the activation of an oncogene or the loss of a tumor-suppressor gene, senescence is induced. P53 was found in premalignant adenoma cells undergoing OIS and is thought to be a regulator of OIS (Bieging et al., 2014). The main function of OIS is to stop abnormal cell proliferation and thus OIS can be seen as an anti-cancer mechanism. In malignant adenoma cells, p53 was not expressed, thus if p53 is expressed adenoma cells are likely to undergo OIS and premalignant adenomas will not develop into malignant adenomas (Collado et al., 2005). Although due to the SASP all senescent cells develop, OIS can also potentially initiate cancer proliferation (Liu et al., 2018).

SASP

The SASP is a phenotype that is seen in all senescent cells, during this phenotype the senescent cells will excrete different soluble and insoluble factors (see figure 2)(Coppé et al., 2010). Important factors for this thesis are; IL-6 and IL-8, which are pro-inflammatory cytokines that have been proven to induce an inflammatory state that can result in more senescence, more inflammation and tumor proliferation (Ortiz-Montero et al., 2017). Next to IL-6 and IL-8, SASP also excretes other factors such as growth factors. In the SASP of fibroblasts, matrix metalloproteinases (MMPs) were found. These MMPs play a role in the modeling of the extracellular matrix (ECM)(see figure 2). Dysregulation of MMPs might be a factor influencing the aging process and the onset of diseases (Freitas-Rodríguez et al., 2017).

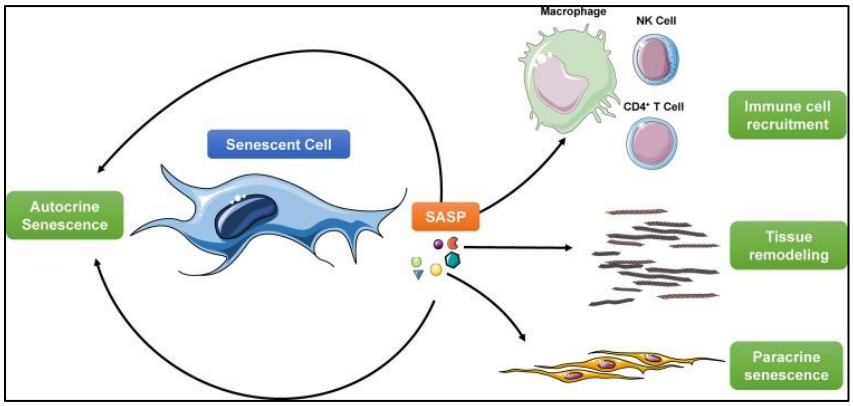

Figure 2. The general effects of the SASP. SASP effects can be beneficial or detrimental depending on the place and time they occur (McHugh & Gil, 2017).

Detrimental SASP

As mentioned earlier, the SASP can have detrimental effects on the environment. The first detrimental effect of the SASP is the chances of cancer relapse after chemotherapy. Senescent cells can promote cancer relapse after chemotherapy. Demaria et al (2017) showed that the removal of senescent cells after chemotherapy resulted in a decrease in cancer relapse. Next to this, senescent cells can promote tumor proliferation as mentioned earlier (Coppé et al., 2006). Krtolica et al. (2001) showed that senescent fibroblasts promoted epithelial cell growth which stimulated premalignant and malignant epithelial cells to become tumors. Furthermore, the continuous excretion of factors by the SASP can lead to the onset of chronic low-grade inflammation which is referred to as 'inflammaging' (Olivieri et

al., 2018). This inflammaging can lead to damage to different types of organs and deregulation of the immune system, senescent cells can activate the immune system and this continuous inflammaging results in a lower clearance of senescent cells which will result in a lasting inflammation and a vicious cycle that is hard to interrupt (Olivieri et al., 2018). Lastly, the SASP can trigger fibrosis and can result in the development of the fatal disease idiopathic pulmonary fibrosis (IPF). In IPF lungs, researchers found different biomarkers for senescence and SASP such as upregulation of CDKN2A which expresses p16 and higher levels of IL-6 (Schafer et al., 2017).

Beneficial SASP

Next to being detrimental, the SASP can be beneficial to the human body (see figure 2). The first beneficial effect of the SASP is the earlier mentioned ability to act as an anti-cancer mechanism (Kang et al., 2011). In addition to this, the SASP can activate both innate and adaptive immune responses. This is one of the mechanisms used by senescent cells to eliminate cancer cells. The SASP recruits the innate immune system to target and kill senescent cells, this also results in an anti-cancer effect (Xue et al., 2007). Furthermore, the SASP also plays a role in the remodeling of tissues, it secretes factors such as platelet-derived growth factor AA to promote wound healing and close wounds. When senescent cells were eliminated in mice models, wound closure was delayed in comparison with mice models where senescent cells were present (Demaria et al., 2014).

Chapter 2: Cardiovascular and Neurodegenerative diseases

Cardiovascular diseases

As mentioned before, CVDs are the most common cause of death worldwide. A condition is called a cardiovascular disease if it affects the heart or blood vessels. There are a lot of different cardiovascular diseases but the top four cardiovascular diseases exist of coronary heart disease, strokes and TIAs, peripheral arterial disease and aortic disease (NHS website, 2022c).

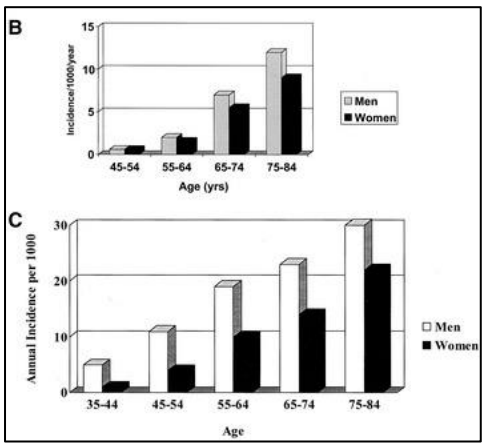

Figure 3. The correlation between age and cardiovascular diseases in men and women of the United States ***B.*** *the incidence of atherothrombotic stroke in men and women* ***C.*** *The incidence of coronary heart disease in men and women. (Lakatta & Levy, 2003).*

Age is one of the major risk factors for the development of cardiovascular diseases (CVDs). In figure 3B and 3C, the correlation between age and the incidences of stroke (figure 3B) and coronary heart disease (figure 3C) is visible in both men and women from the US. In this figure (3B/C) the increased numbers of CVDs with age are clearly visible. In atherosclerosis, there is a narrowing of the arteries due to the building up of plaques existing of fat, blood cells and cholesterol (*Atherosclerosis - What Is Atherosclerosis? | NHLBI, NIH*, 2022). Atherosclerosis is linked to all the four CVDs mentioned earlier in this chapter. During aging there is an increase in Reactive Oxygen Species (ROS), these increased ROS can lead to DNA damage which can lead to an increase in cellular senescence and an

increase in cellular senescence can have detrimental effects as mentioned in chapter 1 (Liguori et al., 2018). Furthermore, the increase in ROS can lead to oxidative stress and an increase in oxidized LDL (ox-LDL). This can lead to the development of atherosclerosis due to endothelial dysfunction and this can cause CVDs (Mitra et al., 2011). Next to an increase in ROS, there is also an increase in the thickening and stiffening of arteries during aging due to changes in compounds of the arterial wall, collagen and elastin (Kohn et al., 2015). Stiffening of arteries plays a role in the development of CVDs, validating once more that aging is a major risk factor for the development of CVDs.

Coronary Heart Disease

Coronary heart disease is a condition in which the blood supply to the heart is blocked or interrupted by a blockage consisting of fatty substances in coronary arteries (NHS website, 2022d). Atherosclerosis is the main cause of coronary heart disease (NHS website, 2022d). Next to atherosclerosis, also hypertension, diabetes, obesity ,smoking and low physical activity are risk factors that influence the onset of coronary heart diseases (Assmann et al., 1999). The occurrence of coronary heart disease is decreasing in the US, Western Europe and Australia but it is increasing in East Europe, Asia and Africa. Making it a disease that is playing right now and will be playing in the future (Sanchis-Gomar et al., 2016).

Strokes and TIA's

A condition is called a stroke if the blood supply to the brain is cut off, there are two different causes of a stroke, there is an ischaemic stroke in which the blood supply is stopped due to a blood clot and there is a haemorrhagic stroke in which a blood vessel supplying the brains bursts and the blood supply is stopped (NHS website, 2022e). A stroke is the second cause of death worldwide, 5.5 million people are killed by a stroke every year and 50% of the survivors of a stroke turn out to become disabled. (Donkor, 2018)

TIA stands for a transient ischaemic attack, and a TIA can be seen as the mild version of a stroke. A condition is called a TIA if there is a temporary disruption of blood supply to part of the brain, but the disruption is not as severe as in a stroke. The effects of a TIA only last a few minutes to hours (NHS website, 2022b). Age is a risk factor for a TIA, people above 55 years old have a higher chance of getting a TIA. TIAs can also be a risk factor for developing a stroke (Khare, 2016). Between 1948 and 2017, Lioutas et al. (2021) discovered that the incidence numbers of a TIA were 1.19 per 1000 persons every year.

Peripheral Arterial Disease

Peripheral arterial disease (PAD), also known as peripheral vascular disease (PVD), shares its cause with coronary heart disease. PAD is caused by a blockage of arteries that supply blood to the leg muscles by build up fatty substances. Atherosclerosis is a risk factor for PAD. PAD can result in the onset of other CVDs such as Coronary Heart Disease or a Stroke (NHS website, 2021). In 2015, 236 million people worldwide suffered from a PAD (Aday & Matsushita, 2021). This number is an estimation as the symptoms of PAD are not that severe and many cases stay undetected.

Aortic Disease

Aortic disease, often known as an abdominal aortic aneurysm (AAA), is a condition in which the aorta is bulged or swollen. If not detected early, the swelling can increase and result in a rupture in the aorta resulting in a life-threatening bleed. Women aged 70+ and men aged 66+ are at risk for developing AAA (NHS website, 2022a). The highest prevalence of AA Ais in the age group between 65 and 74 years (Li et al., 2013). Around 175.000 people die each year from undetected AAA (Howard et al., 2015).

Neurodegenerative diseases

There are multiple neurodegenerative diseases, but the focus will be on the two most common neurodegenerative diseases; Alzheimer's disease and Parkinson's disease. As with CVDs, aging is the major risk factor for the development of neurodegenerative diseases. (Hou et al., 2019).

Alzheimer's Disease

Alzheimer's disease (AD) is the main cause of dementia. AD causes cells in the brain to degenerate, resulting in a decline in brain functions such as thinking and being independent in daily life. Increasing age is a risk factor for AD, next to genetic factors, vascular diseases, and head injuries. There is no cure for AD yet, only two medicines that target the symptoms of AD are on the market. AD is characterized by neurotic plaques and neurofibrillary tangles. During AD, the brain can be seen 'shrinking' as illustrated in figure 4 (Breijyeh & Karaman, 2020). The hippocampus and cerebral cortex are also affected by AD and the ventricles in the brain enlarge during AD. The accumulation of amyloid-beta peptides (Aβ) is the main cause of the neurotic plaques and neurofibrillary tangles (Breijyeh & Karaman, 2020). It is estimated that around 24 million people worldwide are affected by AD, which is expected to double every 20 years until 2040 (Mayeux & Stern, 2012).

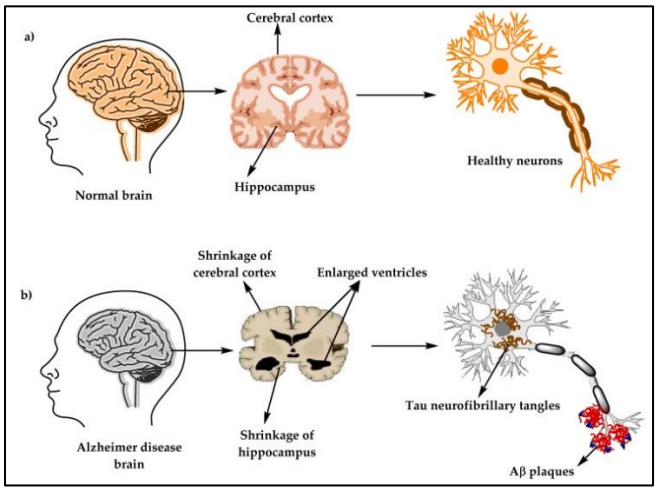

Figure 4. The normal and Alzheimer's disease brain compared. ***A.*** *Normal brain. Healthy neurons and hippocampus are visible.****B,*** *Alzheimer's disease brain. Shrinking of the brain, cerebral cortex and hippocampus are visible together with the enlarged ventricles and next to this the neurotic plaques and neurofibrillary tangles are visible (Breijyeh & Karaman, 2020).*

Parkinson's Disease

After AD, Parkinson's Disease (PD) is the second most common neurodegenerative disease. Age is also a risk factor for PD, due to the aging population, in 2030 it is expected that the cases of PD are increased by 30%. A characteristic of PD is the loss of dopaminergic neurons, this loss of dopaminergic neurons results in different symptoms such as a resting tremor, bradykinesia and postural instability (see figure 5). In the clinic, PD is diagnosed if there is the identification of Lewy bodies (LB) or Lewy neurites (Kouli et al., 2018). α-synuclein is thought to be an important component of the Lewy bodies, in PD there is a mutation in α-synuclein that results in aggregation of α-synuclein into filaments and this causes the formation of more Lewy bodies. PD is therefore categorized as an α-synuclein disease (Spillantini et al., 1997). In PD, the basal ganglia network due to the degeneration of neurons in the substantia nigra is mostly affected (What Is Parkinson's?, 2022).

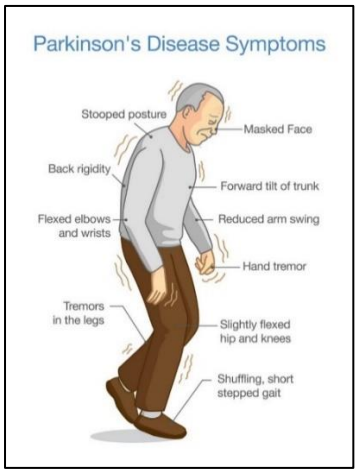

Figure 5. Symptoms of PD. Resting tremor, bradykinesia (reduced arm swing, shuffling, short stepped gait) and postural instability (forward tilt of trunk, back rigidity, stooped posture) are visible (Smith, 2022).

Chapter 3: Senescence in Cardiovascular and Neurodegenerative diseases

As aging is a broad field, the following chapter will not describe the role between aging and senescence, but between the onset of two age-related diseases and senescence to narrow down the focus of this thesis.

Senescence in Cardiovascular diseases

As mentioned before, atherosclerosis is a big risk factor for the development of the most common CVDs namely, coronary heart disease and PAD. Next to this, there is a connection between senescence and the onset of atherosclerosis. Therefore this chapter will look into the connection between senescence and the development of CVDs. There are different types of cells in the cardiovascular system that play a role in senescence and the onset of atherosclerosis and thus the onset of CVDs (see figure 6).

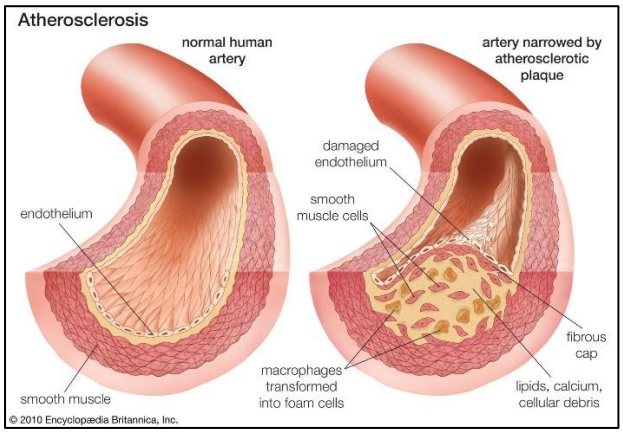

Figure 6. The human endothelium in a healthy state and during atherosclerosis. Human vascular endothelial cells (endothelium in figure) and human vascular smooth muscle cells (smooth muscle in figure) play a role in the onset of atherosclerosis (right side)(Encyclopædia Britannica, Inc., n.d.).

Human Vascular Endothelial Cells

The first type of cells that has a connection with senescence are human vascular endothelial cells (HVECs)(see figure 6). HVECs influence different factors such as nitric oxide (NO), endothelin-1 (ET-1) and Angiotensin II (Wu et al., 2020). When HVECs become senescent, the expression levels of these factors change drastically (Wu et al., 2020). NO is a vasodilator that normally protects against

the development of atherosclerosis. NO prevents the oxidation of LDL in blood vesicles and thereby the formation of the in chapter 2 mentioned ox-LDL. Next to this NO prevents abnormal constriction of the blood vesicle, also called vasospasm (Matthys & Bult, 1997). In HVECs that become senescent, the production of NO is significantly decreased which increases the risk of developing atherosclerosis. ET-1 is an amino-acid peptide that is connected to NO, where NO has vasodilative abilities, ET-1 has a vasoconstrictive effect in arteries. In senescent HVECs, the production of ET-1 is increased (Wu et al., 2020). This increase can lead to hypertension and atherosclerosis, as ET-1 inhibits NO production which leads to an impaired endothelium-dependent relaxation (D'Uscio et al., 2000). Lastly there is angiotensin II, which is a component of the renin-angiotensin-aldosterone system. Angiotensin II is a vasoconstrictor, it can induce endothelial dysfunction, promotes macrophage uptake of ox-LDL and stimulates the production of ROS (Keidar, 1998) (Schmidt-Ott et al., 2000). All of these effects increase the chance of developing atherosclerosis. Senescent HVECs can stimulate the expression level of angiotensin II (Wu et al., 2020).

Human Vascular Smooth Muscle Cells

The second type of cells that is in the cardiovascular system and that is connected to senescence are human vascular smooth muscle cells (HVSMCs)(see figure 6). Senescent HVSMCs can mediate the instability of plaques and play a role in vascular calcification, via levels of bone morphogenetic protein-2 (BMP-2) and excretion of proinflammatory cytokines which are upregulated in senescent HVSMCs (see figure 7)(Wu et al., 2020). BMP-2 plays a role in bone formation by activating osteoblasts, increased levels of BMP-2 promote plaque calcification by the induction of an osteogenic phenotype (see figure 7). Zhang et al.(2015) discovered that in patients with atherosclerosis, plasma levels of BMP-2 were increased. Next to BMP-2, senescent HVSMCs have a SASP that excretes proinflammatory cytokines as mentioned before. These pro-inflammatory cytokines result in chronic low-level inflammation, which can result in the development of atherosclerosis (see figure 7) (Gardner et al., 2015).

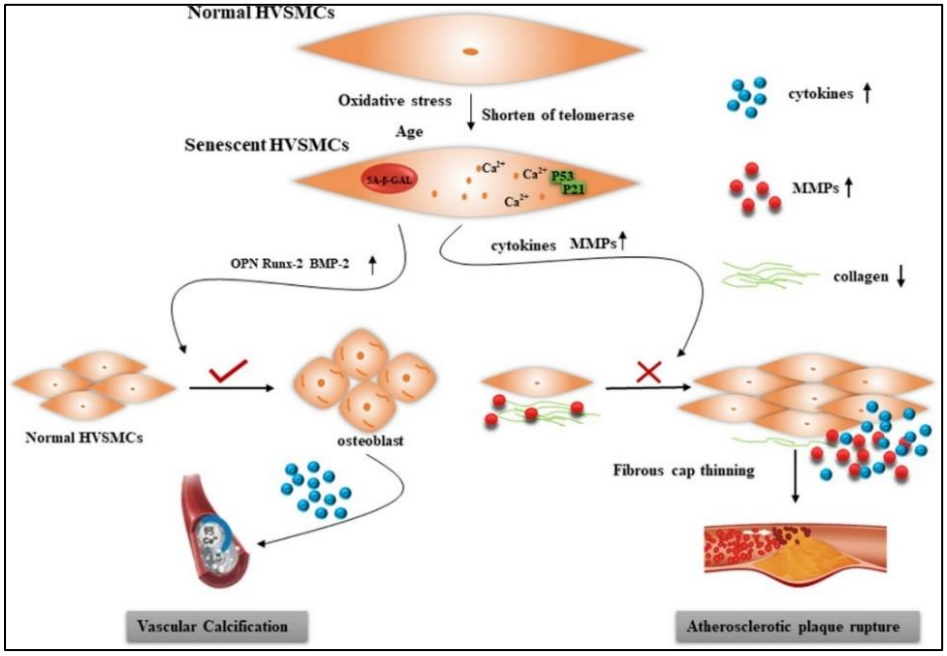

Figure 7. Overview of senescent human vascular smooth muscle cells (HVSMCs).The downstream pathway and influences of BMP-2 (vascular calcification) and proinflammatory cytokines (atherosclerotic plaque rupture) are visible (Wu et al., 2020).

Senescence in Neurodegenerative diseases

There is thought to be a connection between senescence and the onset of neurodegenerative diseases. Most studies suggest that the proinflammatory abilities of senescent cells lead to neuroinflammation that leads to neurodegeneration (Guerrero et al., 2021). During AD and PD, there is neuroinflammation in the environment of the brain. During the onset of AD, this neuroinflammation might be beneficial but when it becomes chronic and AD is progressing, this neuroinflammation is detrimental to the progression of AD (Tailor et al., 2019). As both AD and PD are diseases in the brain, two cell types play a role in regard to senescence. These two cell types are microglia and astrocytes.

Microglia

Microglia are immune cells of the central nervous system. They play a role in the immune protection of the brain. Where neurons cannot divide, microglia can divide and age (Njie et al., 2012). Thus microglia can become senescent. When microglia become senescent they can develop a SASP causing neuroinflammation resulting in the onset of AD and PD (Njie et al., 2012). The IL-6 excretion by the SASP can result in the formation of neurofibrillary tangles (see figure 8) (Streit et al., 2009). Next to this senescent microglia play another role in AD. They are also responsible for the increase in amyloid plaques and thus the development of AD (Wang et al., 2016).

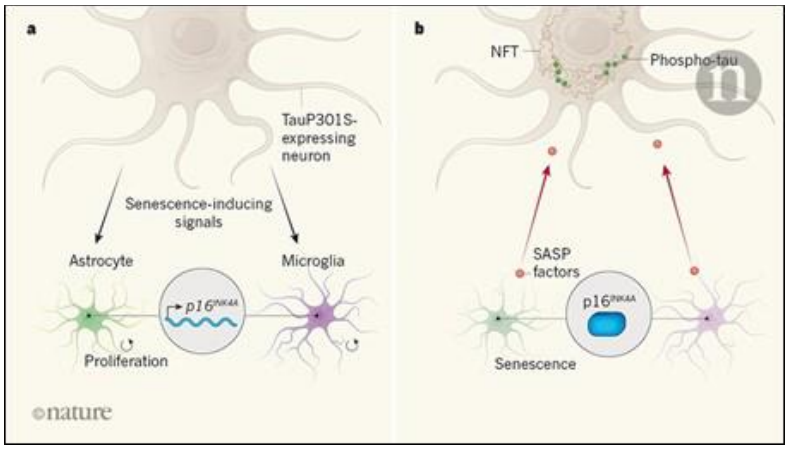

Figure 8. Influence of senescent microglia and astrocytes on neurons. Senescent microglia and astrocytes promote neurofibrillary tangles and amyloid plaques with their SASP resulting in the development of neurodegenerative diseases such as AD and PD (Penney & Tsai, 2018).

Astrocytes

Astrocytes are also cells that are important in the central nervous system, they form around 30% of the cells in the central nervous system. Next to this they form synapses, propagate action potentials and are key players in neuronal development (Liddelow & Barres, 2017). Astrocytes can become senescent. In patients with AD, an increase in $p16^{INK4a}$-positive astrocyte cells has been found (Vazquez-Villaseñor et al., 2019). Also an increase *in vitro* in the production of IL-6 production by senescent astrocytes has been discovered (Bhat et al., 2012). Next to this in the substantia nigra of PD patients, elevated levels of $p16^{INK4a}$ and SASP factors such as IL-6, IL-8 and MMP-3 have been found which could all be traced back to senescent astrocytes (Chinta et al., 2018)

Overall, the connection between AD/PD and senescence is similar, due to the SASP senescent microglia and astrocytes have, neuroinflammation occurs which ultimately leads to neurodegeneration (see figure 9)

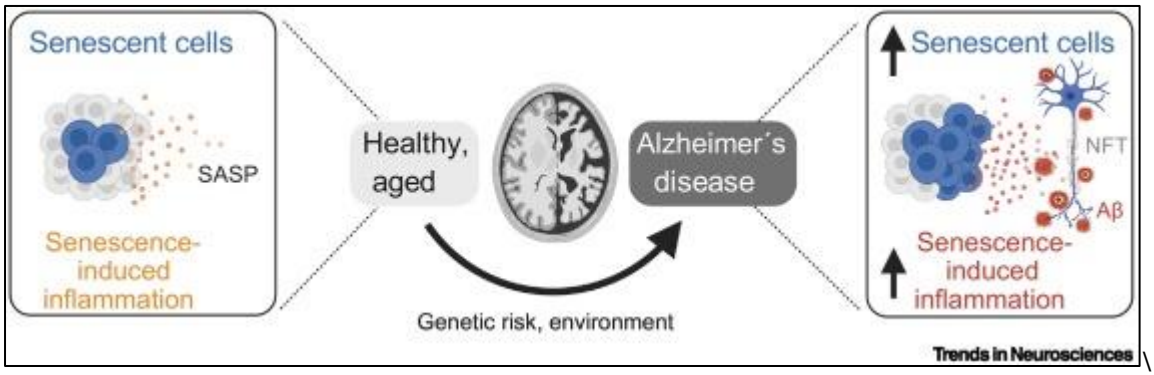

Figure 9. Healthy brain vs Alzheimer's disease brain. In AD, senescent cells induce inflammation resulting in a higher number of senescent cells and neuroinflammation (Guerrero et al., 2021).

Discussion

In this thesis, the role of senescent cells in cardiovascular and neurodegenerative diseases was investigated. Due to the aging population, an increase in senescent cells but also an increase in age-related diseases such as cardiovascular and neurodegenerative diseases will most likely increase. Senescence is seen as a double-edged sword, as it can have beneficial effects such as an anti-cancer mechanism but also detrimental effects such as tumor proliferation. This double-edged sword is due to the SASP senescent cells develop. The SASP can have a beneficial but also detrimental effect on the microenvironment.

To come back to the research question stated in the introduction, senescent cells do play a role in the onset of cardiovascular and neurodegenerative diseases. In CVDs, the biggest connection is between atherosclerosis and senescence. When HVECs become senescent they influence factors such as; NO, ET-1 and angiotensin II (see figure 10). NO levels will decrease in senescent HVECs resulting in an increased risk of developing atherosclerosis. ET-1 levels increase in senescent HVECs and this results in hypertension, a decrease in NO levels and atherosclerosis. Senescent HVECs will react with angiotensin II resulting in endothelial dysfunction, uptake of ox-LDL and stimulation of ROS production. Then, HVSMCs, when HVSMCs become senescent they can influence the calcification of the vascular system and make plaques in atherosclerosis unstable through BMP-2. Senescent HVSMCs also excrete proinflammatory cytokines resulting in chronic low-grade inflammation, increasing the risk for atherosclerosis as seen in figure 10.

Next to CVDs, in neurodegenerative diseases the biggest connection is between two cell types of the central nervous system and senescence, these are microglia and astrocytes. When microglia become senescent they can form neurofibrillary tangles which are detrimental in AD and PD as seen in figure 10. Next to this in senescent microglia and astrocytes, a SASP develops which results in chronic neuroinflammation. This chronic neuroinflammation will result in the development of neurodegenerative diseases such as AD and PD (see figure 10).

Although four connections between CVDs/neurodegenerative diseases and senescence have been found (see figure 10). The exact role of senescence in these age-related diseases is not fully understood yet. This is because senescence and aging are intertwined and there are some vicious cycles (e.g. senescent cells that promote senescence in other cells) that are hard to investigate. Next to this, it is hard to say when a cell is senescent. As there are biomarkers for senescence, e.g. SA-β-GAL, that also mark cells that are not senescent at all (de Mera-Rodríguez et al., 2021). A biomarker that only marks senescent cells is yet to be developed and would be extremely useful. Also, the topic of the CVDs/neurodegenerative diseases and senescence is upcoming and research is just starting in this field when compared to senescence and cancer.

Upcoming in the field of senescence are senolytics, which are compounds that selectively kill senescent cells. As seen in this thesis, senescent cells can have detrimental effects and the removal of senescent cells have improved these detrimental effects in numerous studies (Hickson et al., 2019) (Chang et al., 2015). Further research is needed on the connection between senescence and cardiovascular and neurodegenerative diseases. This research could look into specific types of senolytics that improve cardiovascular and neurodegenerative diseases, such as senolytics aimed at senescent microglia and astrocytes or senescent HVECs and HVSMCs.

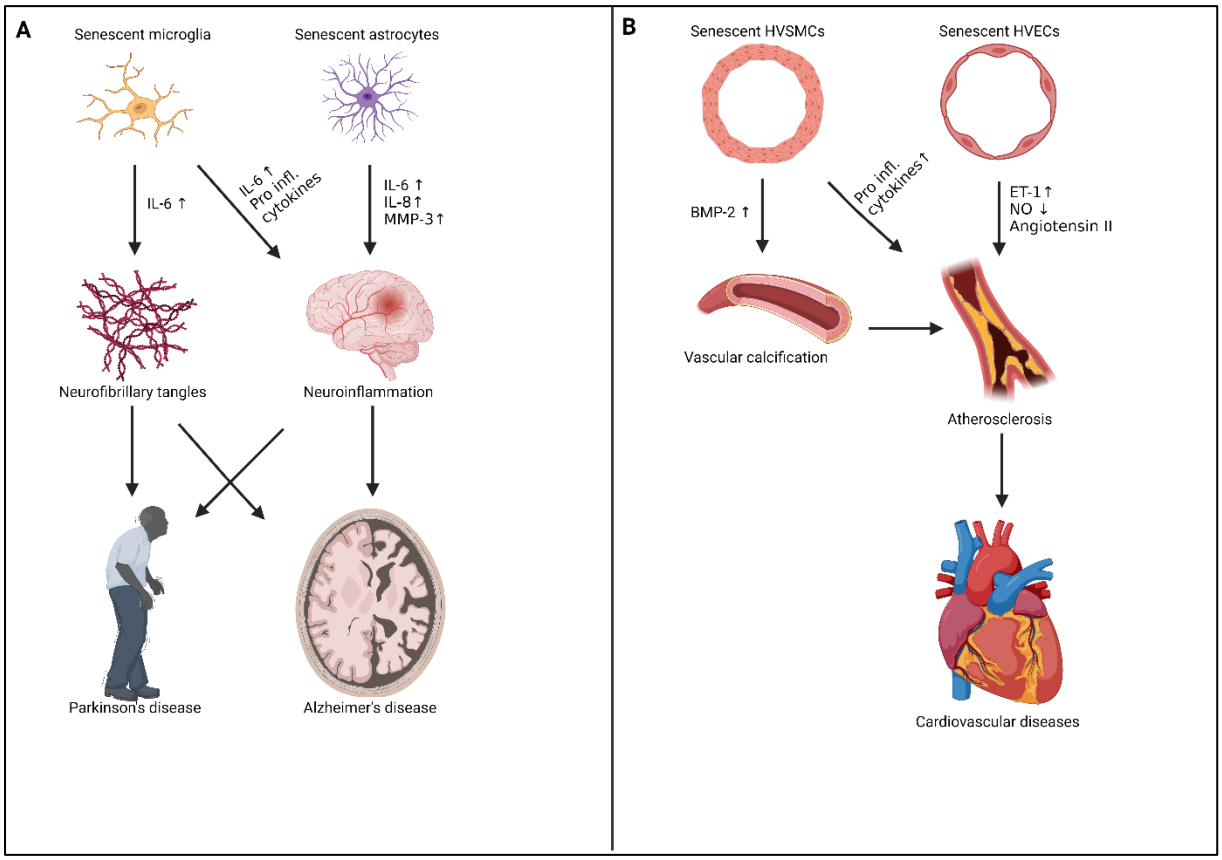

Figure 10. The role of senescence on cardiovascular and neurodegenerative diseases. ***A.*** *Pathway of senescent cells in neurodegenerative diseases.* ***B.*** *Pathway of senescent cells in cardiovascular diseases (made with BioRender.com).*

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References

Aday, A. W., & Matsushita, K. (2021). Epidemiology of Peripheral Artery Disease and Polyvascular Disease. *Circulation Research*, *128*(12), 1818–1832. https://doi.org/10.1161/circresaha.121.318535

Ageing and health. (2021, October 4). WHO. https://www.who.int/news-room/fact-sheets/detail/ageing-and-health

Alzheimer's disease facts and figures. (2021). *Alzheimer's & Dementia*, *17*(3), 327–406. https://doi.org/10.1002/alz.12328

Assmann, G., Cullen, P., Jossa, F., Lewis, B., & Mancini, M. (1999). Coronary Heart Disease: Reducing the Risk. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *19*(8), 1819–1824. https://doi.org/10.1161/01.atv.19.8.1819

Atherosclerosis - What Is Atherosclerosis? | NHLBI, NIH. (2022, March 24). NHLBI. https://www.nhlbi.nih.gov/health/atherosclerosis#:%7E:text=Atherosclerosis%20is%20a%20common%20condition,and%20don't%20know%20it.

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. (2012). Astrocyte Senescence as a Component of Alzheimer's Disease. *PLoS ONE*, *7*(9), e45069. https://doi.org/10.1371/journal.pone.0045069

Bieging, K. T., Mello, S. S., & Attardi, L. D. (2014). Unravelling mechanisms of p53-mediated tumour suppression. *Nature Reviews Cancer*, *14*(5), 359–370. https://doi.org/10.1038/nrc3711

Breijyeh, Z., & Karaman, R. (2020). Comprehensive Review on Alzheimer's Disease: Causes and Treatment. *Molecules*, *25*(24), 5789. https://doi.org/10.3390/molecules25245789

Cardiovascular diseases. (2019, June 11). WHO. https://www.who.int/health-topics/cardiovascular-diseases#tab=tab_1

Chang, J., Wang, Y., Shao, L., Laberge, R. M., Demaria, M., Campisi, J., Janakiraman, K., Sharpless, N. E., Ding, S., Feng, W., Luo, Y., Wang, X., Aykin-Burns, N., Krager, K., Ponnappan, U., Hauer-Jensen, M., Meng, A., & Zhou, D. (2015). Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. *Nature Medicine*, *22*(1), 78–83. https://doi.org/10.1038/nm.4010

Chinta, S. J., Woods, G., Demaria, M., Rane, A., Zou, Y., McQuade, A., Rajagopalan, S., Limbad, C., Madden, D. T., Campisi, J., & Andersen, J. K. (2018). Cellular Senescence Is Induced by the Environmental Neurotoxin Paraquat and Contributes to Neuropathology Linked to Parkinson's Disease. *Cell Reports*, *22*(4), 930–940. https://doi.org/10.1016/j.celrep.2017.12.092

Collado, M., Gil, J., Efeyan, A., Guerra, C., Schuhmacher, A. J., Barradas, M., Benguría, A., Zaballos, A., Flores, J. M., Barbacid, M., Beach, D., & Serrano, M. (2005). Senescence in premalignant tumours. *Nature*, *436*(7051), 642. https://doi.org/10.1038/436642a

Coppé, J. P., Desprez, P. Y., Krtolica, A., & Campisi, J. (2010). The Senescence-Associated Secretory Phenotype: The Dark Side of Tumor Suppression. *Annual Review of Pathology: Mechanisms of Disease*, *5*(1), 99–118. https://doi.org/10.1146/annurev-pathol-121808-102144

Coppé, J. P., Kauser, K., Campisi, J., & Beauséjour, C. M. (2006). Secretion of Vascular Endothelial Growth Factor by Primary Human Fibroblasts at Senescence. *Journal of Biological Chemistry*, *281*(40), 29568–29574. https://doi.org/10.1074/jbc.m603307200

Coppé, J. P., Patil, C. K., Rodier, F., Sun, Y., Muñoz, D. P., Goldstein, J., Nelson, P. S., Desprez, P. Y., & Campisi, J. (2008). Senescence-Associated Secretory Phenotypes Reveal Cell-Nonautonomous

Functions of Oncogenic RAS and the p53 Tumor Suppressor. *PLoS Biology*, *6*(12), e301. https://doi.org/10.1371/journal.pbio.0060301

de Mera-Rodríguez, J. A., Álvarez-Hernán, G., Gañán, Y., Martín-Partido, G., Rodríguez-León, J., & Francisco-Morcillo, J. (2021). Is Senescence-Associated β-Galactosidase a Reliable in vivo Marker of Cellular Senescence During Embryonic Development? *Frontiers in Cell and Developmental Biology*, *9*. https://doi.org/10.3389/fcell.2021.623175

Demaria, M., Ohtani, N., Youssef, S., Rodier, F., Toussaint, W., Mitchell, J., Laberge, R. M., Vijg, J., Van Steeg, H., Dollé, M., Hoeijmakers, J., De Bruin, A., Hara, E., & Campisi, J. (2014). An Essential Role for Senescent Cells in Optimal Wound Healing through Secretion of PDGF-AA. *Developmental Cell*, *31*(6), 722–733. https://doi.org/10.1016/j.devcel.2014.11.012

Demaria, M., O'Leary, M. N., Chang, J., Shao, L., Liu, S., Alimirah, F., Koenig, K., Le, C., Mitin, N., Deal, A. M., Alston, S., Academia, E. C., Kilmarx, S., Valdovinos, A., Wang, B., de Bruin, A., Kennedy, B. K., Melov, S., Zhou, D., . . . Campisi, J. (2017). Cellular Senescence Promotes Adverse Effects of Chemotherapy and Cancer Relapse. *Cancer Discovery*, *7*(2), 165–176. https://doi.org/10.1158/2159-8290.cd-16-0241

Donkor, E. S. (2018). Stroke in the<mml:mrow><mml:msup><mml:mrow><mml:mn mathvariant="normal">21<mml:mrow><mml:mi mathvariant="normal">st</mml:msup>Century: A Snapshot of the Burden, Epidemiology, and Quality of Life. <i>Stroke Research and Treatment, *2018*, 1–10. https://doi.org/10.1155/2018/3238165

D'Uscio, L. V., Barton, M., Shaw, S., & Lüscher, T. F. (2000). Endothelin in Atherosclerosis: Importance of Risk Factors and Therapeutic Implications. *Journal of Cardiovascular Pharmacology*, *35*, S55–S59. https://doi.org/10.1097/00005344-200000002-00013

Encyclopædia Britannica, Inc. (n.d.). *Endothelium* [Illustration]. Www.Britannica.Com. https://www.britannica.com/science/endothelium

Fagagna, F. D. D., Reaper, P. M., Clay-Farrace, L., Fiegler, H., Carr, P., von Zglinicki, T., Saretzki, G., Carter, N. P., & Jackson, S. P. (2003). A DNA damage checkpoint response in telomere-initiated senescence. *Nature*, *426*(6963), 194–198. https://doi.org/10.1038/nature02118

Flatt, T. (2012). A New Definition of Aging? *Frontiers in Genetics*, *3*. https://doi.org/10.3389/fgene.2012.00148

Foy, A. J., & Mandrola, J. M. (2018). Heavy Heart. *Primary Care: Clinics in Office Practice*, *45*(1), 17–24. https://doi.org/10.1016/j.pop.2017.11.002

Freitas-Rodríguez, S., Folgueras, A. R., & López-Otín, C. (2017). The role of matrix metalloproteinases in aging: Tissue remodeling and beyond. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1864*(11), 2015–2025. https://doi.org/10.1016/j.bbamcr.2017.05.007

Gardner, S. E., Humphry, M., Bennett, M. R., & Clarke, M. C. (2015). Senescent Vascular Smooth Muscle Cells Drive Inflammation Through an Interleukin-1α–Dependent Senescence-Associated Secretory Phenotype. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *35*(9), 1963–1974. https://doi.org/10.1161/atvbaha.115.305896

Guerrero, A., de Strooper, B., & Arancibia-Cárcamo, I. L. (2021). Cellular senescence at the crossroads of inflammation and Alzheimer's disease. *Trends in Neurosciences*, *44*(9), 714–727. https://doi.org/10.1016/j.tins.2021.06.007

Hickson, L. J., Langhi Prata, L. G., Bobart, S. A., Evans, T. K., Giorgadze, N., Hashmi, S. K., Herrmann, S. M., Jensen, M. D., Jia, Q., Jordan, K. L., Kellogg, T. A., Khosla, S., Koerber, D. M.,

Lagnado, A. B., Lawson, D. K., LeBrasseur, N. K., Lerman, L. O., McDonald, K. M., McKenzie, T. J., . . . Kirkland, J. L. (2019). Senolytics decrease senescent cells in humans: Preliminary report from a clinical trial of Dasatinib plus Quercetin in individuals with diabetic kidney disease. *EBioMedicine*, *47*, 446–456. https://doi.org/10.1016/j.ebiom.2019.08.069

Hou, Y., Dan, X., Babbar, M., Wei, Y., Hasselbalch, S. G., Croteau, D. L., & Bohr, V. A. (2019). Ageing as a risk factor for neurodegenerative disease. *Nature Reviews Neurology*, *15*(10), 565–581. https://doi.org/10.1038/s41582-019-0244-7

Howard, D. P. J., Banerjee, A., Fairhead, J. F., Handa, A., Silver, L. E., & Rothwell, P. M. (2015). Population-Based Study of Incidence of Acute Abdominal Aortic Aneurysms With Projected Impact of Screening Strategy. *Journal of the American Heart Association*, *4*(8). https://doi.org/10.1161/jaha.115.001926

Jaul, E., & Barron, J. (2017). Age-Related Diseases and Clinical and Public Health Implications for the 85 Years Old and Over Population. *Frontiers in Public Health*, *5*. https://doi.org/10.3389/fpubh.2017.00335

Kang, T. W., Yevsa, T., Woller, N., Hoenicke, L., Wuestefeld, T., Dauch, D., Hohmeyer, A., Gereke, M., Rudalska, R., Potapova, A., Iken, M., Vucur, M., Weiss, S., Heikenwalder, M., Khan, S., Gil, J., Bruder, D., Manns, M., Schirmacher, P., . . . Zender, L. (2011). Senescence surveillance of pre-malignant hepatocytes limits liver cancer development. *Nature*, *479*(7374), 547–551. https://doi.org/10.1038/nature10599

Keidar, S. (1998). Angiotensin, LDL peroxidation and atherosclerosis. *Life Sciences*, *63*(1), 1–11. https://doi.org/10.1016/s0024-3205(98)00014-9

Khare, S. (2016). Risk factors of transient ischemic attack: An overview. *Journal of Mid-Life Health*, *7*(1), 2. https://doi.org/10.4103/0976-7800.179166

Kohn, J. C., Lampi, M. C., & Reinhart-King, C. A. (2015). Age-related vascular stiffening: causes and consequences. *Frontiers in Genetics*, *06*. https://doi.org/10.3389/fgene.2015.00112

Kouli, A., Torsney, K. M., & Kuan, W. L. (2018). Parkinson's Disease: Etiology, Neuropathology, and Pathogenesis. *Parkinson's Disease: Pathogenesis and Clinical Aspects*, 3–26. https://doi.org/10.15586/codonpublications.parkinsonsdisease.2018.ch1

Krtolica, A., Parrinello, S., Lockett, S., Desprez, P. Y., & Campisi, J. (2001). Senescent fibroblasts promote epithelial cell growth and tumorigenesis: A link between cancer and aging. *Proceedings of the National Academy of Sciences*, *98*(21), 12072–12077. https://doi.org/10.1073/pnas.211053698

Kumari, R., & Jat, P. (2021). Mechanisms of Cellular Senescence: Cell Cycle Arrest and Senescence Associated Secretory Phenotype. *Frontiers in Cell and Developmental Biology*, *9*. https://doi.org/10.3389/fcell.2021.645593

Lakatta, E. G., & Levy, D. (2003). Arterial and Cardiac Aging: Major Shareholders in Cardiovascular Disease Enterprises. *Circulation*, *107*(1), 139–146. https://doi.org/10.1161/01.cir.0000048892.83521.58

Li, X., Zhao, G., Zhang, J., Duan, Z., & Xin, S. (2013). Prevalence and Trends of the Abdominal Aortic Aneurysms Epidemic in General Population - A Meta-Analysis. *PLoS ONE*, *8*(12), e81260. https://doi.org/10.1371/journal.pone.0081260

Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D., & Abete, P. (2018). Oxidative stress, aging, and diseases. *Clinical Interventions in Aging*, *Volume 13*, 757–772. https://doi.org/10.2147/cia.s158513

Lioutas, V. A., Ivan, C. S., Himali, J. J., Aparicio, H. J., Leveille, T., Romero, J. R., Beiser, A. S., & Seshadri, S. (2021). Incidence of Transient Ischemic Attack and Association With Long-term Risk of Stroke. *JAMA*, *325*(4), 373. https://doi.org/10.1001/jama.2020.25071

Liu, X. L., Ding, J., & Meng, L. H. (2018). Oncogene-induced senescence: a double edged sword in cancer. *Acta Pharmacologica Sinica*, *39*(10), 1553–1558. https://doi.org/10.1038/aps.2017.198

López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., & Kroemer, G. (2013). The Hallmarks of Aging. *Cell*, *153*(6), 1194–1217. https://doi.org/10.1016/j.cell.2013.05.039

Matthys, K. E., & Bult, H. (1997). Nitric oxide function in atherosclerosis. *Mediators of Inflammation*, *6*(1), 3–21. https://doi.org/10.1080/09629359791875

Mayeux, R., & Stern, Y. (2012). Epidemiology of Alzheimer Disease. *Cold Spring Harbor Perspectives in Medicine*, *2*(8), a006239. https://doi.org/10.1101/cshperspect.a006239

McHugh, D., & Gil, J. (2017). Senescence and aging: Causes, consequences, and therapeutic avenues. *Journal of Cell Biology*, *217*(1), 65–77. https://doi.org/10.1083/jcb.201708092

Mijit, M., Caracciolo, V., Melillo, A., Amicarelli, F., & Giordano, A. (2020). Role of p53 in the Regulation of Cellular Senescence. *Biomolecules*, *10*(3), 420. https://doi.org/10.3390/biom10030420

Mitra, S., Goyal, T., & Mehta, J. L. (2011). Oxidized LDL, LOX-1 and Atherosclerosis. *Cardiovascular Drugs and Therapy*, *25*(5), 419–429. https://doi.org/10.1007/s10557-011-6341-5

Moreno-Blas, D., Gorostieta-Salas, E., Pommer-Alba, A., Muciño-Hernández, G., Gerónimo-Olvera, C., Maciel-Barón, L. A., Konigsberg, M., Massieu, L., & Castro-Obregón, S. (2019). Cortical neurons develop a senescence-like phenotype promoted by dysfunctional autophagy. *Aging*, *11*(16), 6175–6198. https://doi.org/10.18632/aging.102181

Mylonas, A., & O'Loghlen, A. (2022). Cellular Senescence and Ageing: Mechanisms and Interventions. *Frontiers in Aging*, *3*. https://doi.org/10.3389/fragi.2022.866718

Nagano, T., Nakano, M., Nakashima, A., Onishi, K., Yamao, S., Enari, M., Kikkawa, U., & Kamada, S. (2016). Identification of cellular senescence-specific genes by comparative transcriptomics. *Scientific Reports*, *6*(1). https://doi.org/10.1038/srep31758

NHS website. (2021, November 18). *Peripheral arterial disease (PAD)*. Nhs.Uk. https://www.nhs.uk/conditions/peripheral-arterial-disease-pad/

NHS website. (2022a, May 30). *Abdominal aortic aneurysm*. Nhs.Uk. https://www.nhs.uk/conditions/abdominal-aortic-aneurysm/

NHS website. (2022b, June 14). *Transient ischaemic attack (TIA)*. Nhs.Uk. https://www.nhs.uk/conditions/transient-ischaemic-attack-tia/

NHS website. (2022c, June 24). *Cardiovascular disease*. Nhs.Uk. https://www.nhs.uk/conditions/cardiovascular-disease/

NHS website. (2022d, June 28). *Coronary heart disease*. Nhs.Uk. https://www.nhs.uk/conditions/coronary-heart-disease/#:%7E:text=Coronary%20heart%20disease%20is%20the,furred%20up%20with%20fatty%20deposits.

NHS website. (2022e, June 28). *Stroke*. Nhs.Uk. https://www.nhs.uk/conditions/stroke/

Niedernhofer, L. J. (2019). Endogenous DNA damage as a driver of senescence and aging. *The FASEB Journal*, *33*(S1). https://doi.org/10.1096/fasebj.2019.33.1_supplement.342.2

Njie, E. G., Boelen, E., Stassen, F. R., Steinbusch, H. W., Borchelt, D. R., & Streit, W. J. (2012). Ex vivo cultures of microglia from young and aged rodent brain reveal age-related changes in microglial function. *Neurobiology of Aging*, *33*(1), 195.e1-195.e12. https://doi.org/10.1016/j.neurobiolaging.2010.05.008

Olivieri, F., Prattichizzo, F., Grillari, J., & Balistreri, C. R. (2018). Cellular Senescence and Inflammaging in Age-Related Diseases. *Mediators of Inflammation*, *2018*, 1–6. https://doi.org/10.1155/2018/9076485

Ortiz-Montero, P., Londoño-Vallejo, A., & Vernot, J. P. (2017). Senescence-associated IL-6 and IL-8 cytokines induce a self- and cross-reinforced senescence/inflammatory milieu strengthening tumorigenic capabilities in the MCF-7 breast cancer cell line. *Cell Communication and Signaling*, *15*(1). https://doi.org/10.1186/s12964-017-0172-3

Ouchi, R., Okabe, S., Migita, T., Nakano, I., & Seimiya, H. (2016). Senescence from glioma stem cell differentiation promotes tumor growth. *Biochemical and Biophysical Research Communications*, *470*(2), 275–281. https://doi.org/10.1016/j.bbrc.2016.01.071

Penney, J., & Tsai, L. H. (2018). Elimination of senescent cells prevents neurodegeneration in mice. *Nature*, *562*(7728), 503–504. https://doi.org/10.1038/d41586-018-06677-7

Pérez-Sayáns, M., Suárez-Peñaranda, J. M., Padín-Iruegas, M. E., Gayoso-Diz, P., Reis-De Almeida, M., Barros-Angueira, F., Gándara-Vila, P., Blanco-Carrión, A., & García-García, A. (2015). The Loss of p16 Expression Worsens the Prognosis of OSCC. *Applied Immunohistochemistry & Molecular Morphology*, *23*(10), 724–732. https://doi.org/10.1097/pai.0000000000000133

Sanchis-Gomar, F., Perez-Quilis, C., Leischik, R., & Lucia, A. (2016). Epidemiology of coronary heart disease and acute coronary syndrome. *Annals of Translational Medicine*, *4*(13), 256. https://doi.org/10.21037/atm.2016.06.33

Schafer, M. J., White, T. A., Iijima, K., Haak, A. J., Ligresti, G., Atkinson, E. J., Oberg, A. L., Birch, J., Salmonowicz, H., Zhu, Y., Mazula, D. L., Brooks, R. W., Fuhrmann-Stroissnigg, H., Pirtskhalava, T., Prakash, Y. S., Tchkonia, T., Robbins, P. D., Aubry, M. C., Passos, J. F., . . . LeBrasseur, N. K. (2017). Cellular senescence mediates fibrotic pulmonary disease. *Nature Communications*, *8*(1). https://doi.org/10.1038/ncomms14532

Schmidt-Ott, K. M., Kagiyama, S., & Phillips, M. (2000). The multiple actions of angiotensin II in atherosclerosis. *Regulatory Peptides*, *93*(1–3), 65–77. https://doi.org/10.1016/s0167-0115(00)00178-6

Smith, J. (2022, June 24). *Axovant's Parkinson's Disease Gene Therapy Clinical Trial Launched in UK*. Labiotech.Eu. https://www.labiotech.eu/trends-news/axovant-parkinsons-disease-gene/

Sopina, E., Spackman, E., Martikainen, J., Waldemar, G., & Sørensen, J. (2018). Long-term medical costs of Alzheimer's disease: matched cohort analysis. *The European Journal of Health Economics*, *20*(3), 333–342. https://doi.org/10.1007/s10198-018-1004-0

Spillantini, M. G., Schmidt, M. L., Lee, V. M. Y., Trojanowski, J. Q., Jakes, R., & Goedert, M. (1997). α-Synuclein in Lewy bodies. *Nature*, *388*(6645), 839–840. https://doi.org/10.1038/42166

Streit, W. J., Braak, H., Xue, Q. S., & Bechmann, I. (2009). Dystrophic (senescent) rather than activated microglial cells are associated with tau pathology and likely precede neurodegeneration in Alzheimer's disease. *Acta Neuropathologica*, *118*(4), 475–485. https://doi.org/10.1007/s00401-009-0556-6

Tailor, B., Pilozzi, A., & Huang, X. (2019). Contributing Factors of Neurodegeneration in Alzheimer's Disease. *Alzheimer's Disease*, 69–84. https://doi.org/10.15586/alzheimersdisease.2019.ch5

Vazquez-Villaseñor, I., Garwood, C. J., Heath, P. R., Simpson, J. E., Ince, P. G., & Wharton, S. B. (2019). Expression of p16 and p21 in the frontal association cortex of ALS / MND brains suggests neuronal cell cycle dysregulation and astrocyte senescence in early stages of the disease. *Neuropathology and Applied Neurobiology*, *46*(2), 171–185. https://doi.org/10.1111/nan.12559

Wang, Y., Ulland, T. K., Ulrich, J. D., Song, W., Tzaferis, J. A., Hole, J. T., Yuan, P., Mahan, T. E., Shi, Y., Gilfillan, S., Cella, M., Grutzendler, J., DeMattos, R. B., Cirrito, J. R., Holtzman, D. M., & Colonna, M. (2016). TREM2-mediated early microglial response limits diffusion and toxicity of amyloid plaques. *Journal of Experimental Medicine*, *213*(5), 667–675. https://doi.org/10.1084/jem.20151948

What Is Parkinson's? (2022). Parkinson's Foundation. https://www.parkinson.org/understanding-parkinsons/what-is-parkinsons

Wu, C. M., Zheng, L., Wang, Q., & Hu, Y. W. (2020). The emerging role of cell senescence in atherosclerosis. *Clinical Chemistry and Laboratory Medicine (CCLM)*, *59*(1), 27–38. https://doi.org/10.1515/cclm-2020-0601

Xue, W., Zender, L., Miething, C., Dickins, R. A., Hernando, E., Krizhanovsky, V., Cordon-Cardo, C., & Lowe, S. W. (2007). Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. *Nature*, *445*(7128), 656–660. https://doi.org/10.1038/nature05529

Zhang, M., Sara, J. D., Wang, F. L., Liu, L. P., Su, L. X., Zhe, J., Wu, X., & Liu, J. H. (2015). Increased plasma BMP-2 levels are associated with atherosclerosis burden and coronary calcification in type 2 diabetic patients. *Cardiovascular Diabetology*, *14*(1). https://doi.org/10.1186/s12933-015-0214-3