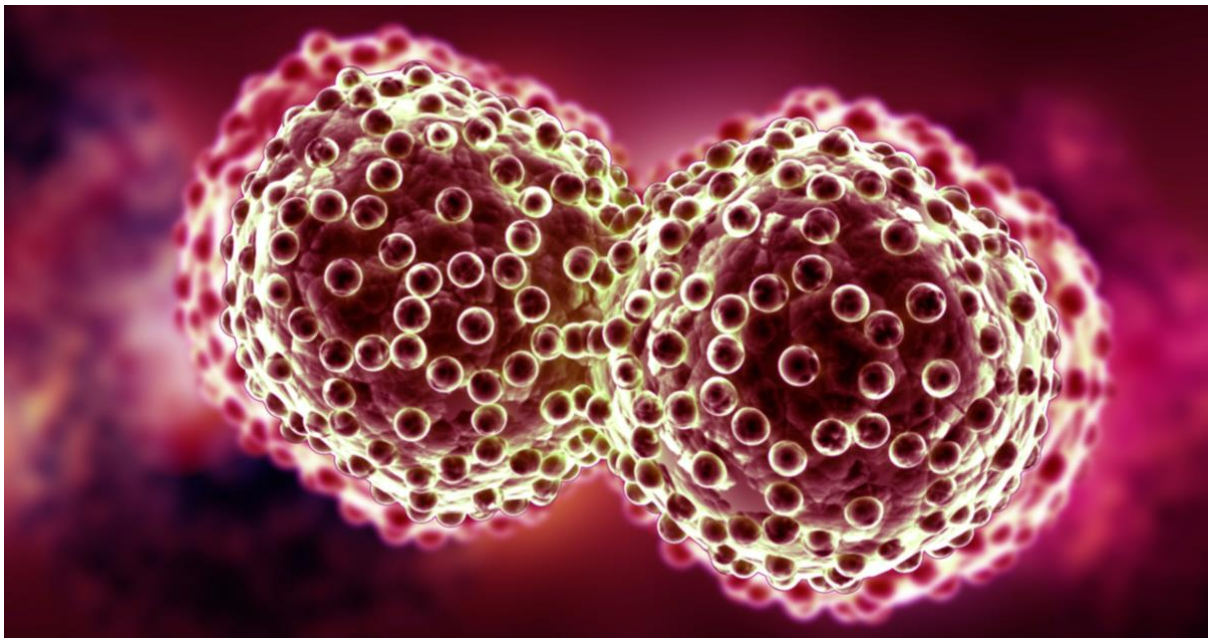
The controversial role of cellular senescence in the development of cancer



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Abstract

Cellular senescence is a hallmark of aging characterized by a stable, irreversible cell cycle arrest, which can be induced by various stimuli. For example, telomere shortening, DNA damage, and oncogenic signaling. Key features of senescent cells are the enhancement of gene expression, morphological changes, and chromatin reorganization. Numerous studies have been demonstrating the paradoxical role of cellular senescence. These studies have both shown how senescence might have a role in the development of cancer and its prevention of it. At first, senescence was thought to be a potent anticancer mechanism, because when tumor cells enter a senescent state they stop being able to proliferate. Also, the feature senescent-associated secretory phenotype, better known as SASP is known to induce senescence and cause the stop of the proliferation of tumor cells. However, growing evidence suggests that this characteristic of cellular senescence, the SASP, is involved in the positive promotion of tumor cells. In this article, the controversial role of cellular senescence in the development of cancer is discussed.

Introduction **4**

Cancer **5**

Hallmarks of cancer 5

Cancer treatment 7

Cancer and cellular senescence **7**

Cellular senescence **7**

Inducers of senescence 8

Pathways of senescence 9

Characteristics of cellular senescence 10

Cellular senescence as a tumor suppressant **11**

Cellular senescence as a tumor promoter **12**

Discussion **14**

References **16**

Introduction

As healthcare is getting more improved, the human lifespan is also dramatically increased. This increase in lifespan, however, also increases the cases of age-related diseases. Cancer is a well-known age-related disease that is a global health problem. There are annually millions of cancer-related deaths worldwide. In 2020, 19.3 million cancer cases and 10.0 million cancer-related deaths were registered (Sung et al., 2021). As the number of cancer cases is exceedingly increased, much research is done on new potential treatments against cancer. The amount of cancer cases increases substantially in aging. The biggest risk factor for cancer is aging (Hyde, 2018).

Cellular senescence is considered an important hallmark of both aging and cancer. When cells become senescent the cell cycle is arrested. This senescent state can be induced by developmental signals and various intrinsic and extrinsic stimuli, such as DNA damage, telomere shortening, and oncogenic signaling (Kumari et al., 2021). In senescent cells, the cell cycle is arrested and is irreversible. Senescent cells alter metabolic activity and remain viable and resistant to apoptosis (Ryu et al., 2007; Hampel et al., 2004). Characteristics of senescent cells are macromolecular damage, increased lysosomal mass, and the development of the senescence-associated secretory phenotype (SASP) complex. Moreover, changes in the structure and morphology occur when senescence is induced. The cells become more flattened and enlarged and the vacuoles are increased. The composition of the plasma membrane is altered, and the nucleus is remarkably enlarged (Campisi et al., 2007). The changes in the characteristics of the senescent cell cause the implementation of various aspects of cellular senescence, such as the development of the SASP phenotype and growth arrest.

Senescence is considered a crucial biological mechanism involved in the suppressants of tumors. The main anti-tumor effect of senescent cells is the arrested cell cycle in tumor cells. For example, oncogene-induced senescence acts as a protective barrier for the prevention of the proliferation of transformed cells (Collado et al., 2010). Different studies have shown that cellular senescence limits cancer progression, but blocks apoptosis as a consequence of telomere dysfunction in cancer cells (Cosme-Blanco et al., 2007). Interestingly, different SASP components enhance or induce growth arrest in paracrine and autocrine manners These component also play a role in recruiting immune cells, propagating senescence, and producing a pro-inflammatory environment, which inhibits the progression of cancer (Capece et al., 2018). The SASP mechanism can stimulate the immune system to target different types of malignant cells to inhibit the progression of tumor cells (Thiers, 2008).

However, despite its anti-tumor properties, cellular senescence acts as a double-edged sword with both beneficial and detrimental effects on the health of humans. This

depends on the trigger, pathway, and stimuli involved. For example, senescence was thought to be a potent anticancer mechanism because when senescence is induced**,** cancer cells lose their most harmful characteristic, namely, they stop proliferating (Kuilman et al., 2008). However numerous studies suggest that senescence plays a negative role in treating cancer. These studies show growing evidence that the accumulation of senescent cells has a role in tumor progression (Wiley, 2020). First, the senescence-associated secretory phenotype is thought to directly or indirectly promote the growth of tumor cells, vascularization, invasion, and tumor metastasis (Davalos et al., 2010). Second, senescent fibroblasts stimulate malignant and premalignant tumors from epithelial cells (Krtolica et al., 2001). Third, cellular senescence is thought to have an increasing effect on angiogenesis. with the reprogramming of malignant cells and stem-cell-like properties (Schmitt, 2018).

The main research question that this article will be focussing on is: 'What is the controversial role of cellular senescence in the development of cancer?'

Cancer

Hallmarks of cancer

The principle of cancer is characterized as the uncontrolled proliferation of cells. Cancer cells also develop acquisitions of metastatic properties. There are six hallmarks of cancer that collectively dictate the growth of malignant cells (Hanahan & Weinberg, 2011).

1. Self-sufficiency in the generation of growth signals

The change in growth signal autonomy was the first of the six alterations to be discovered by researchers in cancer cells (Hanahan & Weinberg, 2011). Normal cells require growth signals before they can start proliferating. These growth signals usually are transmitted in the cell via receptors that are embedded in the transmembrane. These receptors bind to distinctive classes of molecules, such as extracellular matrix components, cell-to-cell adhesion molecules, and diffusible growth factors (Hanahan et al., 2000). However, tumor cells generate signals that stimulate growth. This results in the liberation from dependence on stimulation from signals of their normal microenvironment. This will eventually lead to the disruption of important homeostatic mechanisms which operates the behavior of cell types within the human tissue(Hanahan et al., 2000).

2. Insensitivity to antiproliferative signals

In normal tissue numerous anti-growth signals are present. These anti-proliferative signals are present to maintain tissue homeostasis and quiescence. These signals include immobilized inhibitors and soluble growth inhibitors embedded on the surfaces of neighboring cells and in the extracellular matrix (Hanahan et al., 2000). As cancer cells are proliferating constantly they evade these anti-proliferative signals. This is done via the disruption of the retinoblastoma protein pathway (Hanahan et al., 2000).

3. Avoiding apoptosis

A characteristic of tumor cells is the rapidly expanding number of cells. Not only is this increase in cell number influenced by the rate of proliferation of cells. Also apoptosis, which is programmed cell death, represents a major role in the number of cells in the tumor cell population. Apoptosis involves a series of cellular processes that activate the formation of caspases leading to cell death (Fernald et al., 2013). Numerous studies suggest that in cultured cells and mouse models, tumor cells acquire an alteration towards apoptosis resistance (Hanahan et al., 2000).

4. Unlimited replicative potential

The previously mentioned capabilities are; self-sufficiency in the generation of growth signals, insensitivity to anti-growth signals and at last: avoiding apoptosis. This results in an alteration in the cell's growth program in its environment. Most tumor cells appear to be immortalized, with the ability to proliferate without limitation (Wright et al., 1989). This suggests that limited replication is essential for developing the malignant growth state (Hanahan et al., 2000).

5. Sustained formation of blood vessels

As new tissue is formed new blood vessels are made, this process is called angiogenesis. This is crucial, as nutrients and oxygen are essential for the formation of new cells. It seems plausible that the cells that are proliferating would have the ability to promote the growth of blood vessels, because of the dependence on neighboring capillaries (Wright et al., 1989). However, studies suggest that cells with irregular proliferation lack the ability to activate angiogenesis This curtails the capability of expansion (Hanahan et al., 2000).

6. Metastasis and tissue invasion

A characteristic of cancer cells is the ability to travel to distant sites to find a new colony. Cancer cells also have the ability to invade adjacent tissues. Metastasis, the ability to travel to distant sites, is causing 90% of all cancer-related death (Sporn, 1996). Invasion and metastasis enable the cancer cells to colonize new tissues in the body by escaping from their

primary tumor mass (Hanahan et al., 2000). This metastasis and invasion depend on the other five altered capabilities.

Cancer treatment

As cancer is highly heterogeneous, meaning there is a high degree of diversity between types of cancer cells. Due to this heterogeneity, there is not a general type of treatment available to cure all cancers. Different factors are necessary for the development of cancer treatment. As cancer is a very complex type of disease. Due to these previously noted hallmarks it has to be deeply understood to be able to design a specific therapy. The two most common types of cancer treatment are listed below:

- Chemotherapy
    - In chemotherapy, DNA damaging reagents are used in order to kill a large number of cancer cells. This therapy targets rapidly proliferating cells however, also rapidly proliferating healthy cells are targeted when using this treatment leading to serious side effects such as, infection or fatique (*How Does Chemo Work?* | *Types of Chemotherapy*, 2019).
- Radiation therapy
    - During radiation therapy, small breaks of the DNA of the cancer cell is induced, causing the cancer cells to stop proliferating eventually leading towards cell death.Neighbouring cells can also be targeted by this treatment causing normal healthy cells to die (*How Does Chemo Work?* | *Types of Chemotherapy*, 2019).

Cancer and cellular senescence

In the tumor microenvironment (TME) in cancer, senescent cells frequently arise. This is due to the intrinsic and extrinsic stimuli of how senescent cells could be induced. DNA damage is one of the stimuli which causes cancer. In these precancerous cells the DNA replication is disregulated resulting from different oncogenes, such as RAS and RAF (Serrano et al., 1997). These precancerous cells show the same phenotype as cells which entered a senescent state thought different kinds of stimuli (Serrano et al., 1997)

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Cellular senescence

Cellular senescence was discovered in the 1960s by Hayflick and Moorhead (Ogrunc et al., 2011). They discovered that human fibroblasts had a limited ability to proliferate. Senescence is implicated in different processes such as aging and the prevention and development of

cancer. The accumulation of cells that are senescent has been associated with numerous age-related diseases, such as cardiovascular diseases (Wang et al., 2016), Parkinson's disease (Calabrese et al., 2018), and cancer (Davalos et al., 2010). When cells enter a senescent state, they fail to launch replication but remain metabolically active (Krtolica et al., 2001). Senescent cells have the ability to secrete proinflammatory molecules. This is highlighted because this characteristic is known to promote the formation of cancer cells (Borghesan et al., 2019).

Inducers of senescence

Senescence can be induced by various intrinsic and extrinsic factors, such as oxidative stress, DNA damage from radiotherapy and chemotherapy, oncogenic signaling, and replicative stress, called replicative senescence. Non-replicative inductors are named premature senescence (Toussaint et al., 2006). When the senescent trigger is removed, senescent cells will not re-enter the proliferating state.

DNA damage is the most common inductor of senescence (D'Adda Di Fagagna, 2008). Typically, the DNA damage response is activated by the breakage of the DNA backbone. An inflictor that is known to induce DNA damage is UV radiation (Rastogi et al., 2010). This will lead to the breakage of the double-stranded DNA (D'Adda Di Fagagna, 2008). DNA damage can be induced by topoisomerase inhibitors, ionizing radiation, and other factors (Robles et al., 1998). After persistent DNA damage, these double-stranded breaks promote the secretion of different kinds of inflammatory cytokines, such as IL-6 (Rodier et al., 2009).

Telomere shortening results in replicative senescence. Telomeres, repetitive DNA sections at the end of linear chromosomes that protect the chromosomes from fusion or degradation are shortened after each round of replication. This malfunction happens due to the enzyme DNA polymerase not being able to replicate the end of the linear DNA, which results in the loss of 50-200 base pairs after each cycle (Beausejour, 2003). Consequently, Telomeres limit the proliferation of the cell with every division, as it functions as a biological clock (Hayflick limit) (Wojtyla et al., 2010). When telomeres are shortened and become dysfunctional, a DNA damage response (DDR) occurs. Uncapped chromosome ends are recognized as a double-strand break (DBS) by DNA repair machinery. In order to avoid instability of the genome a senescence response is induced (Tusell et al., 2010).

Oncogenes or the loss of tumor suppressors are independent of telomeric length and induce so-called oncogene-induced senescence (OIS) (Coppé et al., 2008; Shamma et al., 2009). The overexpression of oncogenes, such as RAS, inflicts a cell cycle arrest in the G1 phase. Which is caused by the MAPK cascade, a mechanism crucial in cellular processes, such as proliferation (Plotnikov et al., 2011). Moreover, premature senescence is promoted by the activation of the tumor suppressor proteins p16 and p53 (Serrano et al., 1997). Different

oncogenes that also are capable of inducing oncogene-induced senescence are; BRAF, RAF, ARF, CDC6, MOS, and cyclin E (Lin et al., 1998).

Pathways of senescence

Cellular senescence is induced via two pathways, the p16 / pRB pathway, and the p53 / p21 pathway (Rovillain et al., 2011). The first pathway is involved in the early induction of senescence. For example, via exposure to a certain oncogene. This early induction is named the first plateau, telomere-independent senescence, or the mortality phase 0 (M0). In this M0 phase, senescent cells express an increased concentration of p16. Furthermore, an increase in senescence- ß-galactosidase activity, and morphologically the cell is enlarged (Abbadie et al., 2017). Interestingly, when the activation of p16 is silenced, cells will start dividing in spite of induced DNA damage, and cells will leave the M0 phase (Ben-Porath et al., 2005). Senescent cells in the M0 phase that undergo certain mutations have the ability to escape the arrested state, which causes the formation of clones of these cells (Shay et al., 1991).

When this occurs, senescence will be induced by the shortening of telomeres. This phase is called the second plateau or mortal phase 1 (M1) and is triggered by the p53 / p21 pathway. The cells in this phase have the same characteristics as cells in the M0 phase. Additionally, when the activation of p53 is silenced, cells will escape the M1 phase and are still able to divide until they reach the final phase, mortality phase 2 (M2). This phase is characterized by instability of the genome, causing cell death. However, when inactivation of M2 controlling genes occurs, cells in the M2 phase become immortal (Campisi et al., 2007) (Figure 1). Both these pathways induce the activation of NF-κβ, which induces the upregulation of two cytokines that are crucial for the induction of senescence, IL-6, and Il-8 (Acosta et al., 2013).

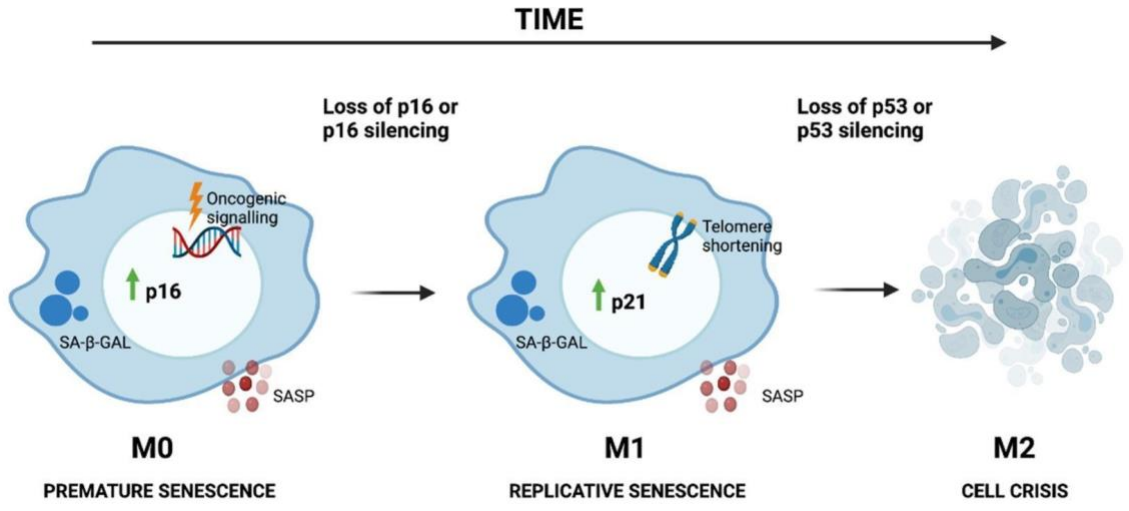

Figure 1. When cells are exposed to oncogenic signaling cells enter the M0 phase. In this phase, the cells express an increased level of p16. When p16 expression is silenced, cells will enter the M1 phase when telomeres are

shortened. In this phase, the cells express an increased level of p21. When p53 expression is silenced, cells enter the M2 phase, causing cell crisis and eventually cell death.

Characteristics of cellular senescence

Senescent cells have certain features that characterize the senescent phenotype. A combination of markers and characteristics is used to identify senescent cells in vivo, considering none of these features are exclusive to senescent cells.

Morphologically, senescent cells exhibit an enlarged cellular size as they continue to be metabolically active. Furthermore, the cell becomes more flattened, an accumulation of stress granules occurs and vacuoles start forming (Campisi, 2018). Senescent cells have an increase in lysosomal mass (Lee et al., 2006). This characteristic is used as a marker to detect senescent cells. Due to this increase, an overexpression of β-galactosidase is used to detect this marker (Dimri et al., 1995). Also, an irregular nuclear envelope and enlarged nuclei are present in senescent cells. Changes in chromosome distribution and condensation also are characteristics of cellular senescence.

Moreover, gene expression is also altered in senescent cells. A characteristic of senescent cells is the upregulation of certain tumor suppressor genes (Campisi, 2001). In order to identify senescent cells, the upregulation of p16 is a well-known marker for this identification (Abbadie et al., 2017). This protein is rarely expressed in normal cells, but in aging it becomes more abundant (Campisi & D'Adda Di Fagagna, 2007). Due to this upregulation of certain genes for example, Ras and Raf genes and certain molecules such as, p16 and p21 get secreted by senescent cells (Lin et al., 1998; Serrano et al., 1997). For example, growth factors, immune modulators, and inflammatory cytokines. The secretion of these molecules is a common characteristic of senescent cells and is called senescent-associated-secretory-phenotype, better known as SASP (Campisi & D'Adda Di Fagagna, 2007).

In senescent cells, chromatin reorganization takes place, called senescence-associated-heterochromatin-foci (SAHF). Chromatin gets reorganized in foci, where proliferative genes are silenced (Sharpless & Sherr, 2015). Senescent-associated DNA damage-foci (SDF) can also be recognized as a marker in senescent cells with accumulated DNA damage, such as the phosphorylation of 53BP1 or ɣH2AX (Mah et al., 2010; Di Micco et al., 2006)). However, the phosphorylation of ɣH2AX is also a present damaged DNA part independent of senescence, so it is not a specific marker of cellular senescence (Dodig et al., 2019).

Cellular senescence as a tumor suppressant

Recent study showed that when DNA damage is induced, for example by IR-radiation, the p53/p21 pathway is activated which induces senescence (Qin et al., 2018). In this study, Nutilin 3 was used in order to inhibit p53. After inducing DNA damage MicroRNA-34 (miR-34A) expression was upregulated (Qin et al., 2018). miR-34A is a responsive miRNA involved in the regulation of senescence induction (Hermeking, 2007). The study showed the increased expression of this miRNA suggests the promotion of senescence induction (Qin et al., 2018).

SASP is a feature characterized by senescent cells. Different components of the SASP complex enhance or induce growth arrest induced by cellular senescence. These secreted molecules play a key role in recruiting immune cells, propagating senescence, and producing an inflammatory environment. All these factors cause the inhibition of tumor progression (Capece et al., 2018). When the oncogene RAF is activated IL-6 and IL-8 are upregulated. These cytokines play a crucial role in the maintenance of the SASP response. This activation leads to the promotion of cellular senescence (Kuilman et al., 2008; Acosta et al., 2008). When these cytokines are blocked, caspase-1 prevents the induction of SASP in senescent cells (Groß et al., 2012). Meaning that IL-1 plays a crucial role in the maintenance of senescence and the induction of the inflammatory cytokines of the SASP mechanism, leading to the suppression of tumor growth.

Furthermore, a crucial feature of SASP is the communication with extracellular matrix and neighboring cells, which has been known to have tumor-suppressing effects. When DNA damage is monitored by cells the pro-inflammatory cytokine IL-8 is activated and transmitted between other cell types (Acosta et al., 2013). In different in vivo models, for example mouse models, SASP showed to have the ability to induce paracrine senescence. Different SASP components are identified that play a crucial role in this process, such as vascular endothelial growth factor (VEGF) and transforming growth factor-β (TGF-β) (Acosta et al., 2013). As these models show that SASP is able to induce paracrine senescence, it is conceivable that it promotes the suppression of tumor cells. (Pérez-Mancera et al., 2014).

Additionally, another key feature of the SASP mechanism is the activation of immune surveillance, which shows to have an anti-proliferative effect on tumor cells (Thiers, 2008). SASP has the ability to target premalignant and malignant cells, suppressing the progression of tumor growth by stimulating the immune system. The tumor suppressor protein p53 activates the induction of senescence in malignant hepatocytes, contributing to the clearance of tumor cells through inflammatory cytokines and SASP-mediated differentiation (Thiers, 2008). An anti-tumor barrier that is linked to senescence is the ability of pre-malignant senescent hepatocytes to secrete cytokines and chemicals, which are being cleared by immune cells that infiltrated the liver (Kang et al., 2011). The recruitment of natural killer cells

is also a key characteristic of the SASP complex. The recruitment of these cells alters the polarization of macrophages suppressing tumorigenesis and eliminating senescence-induced tumor cells (Figure 2).

Cellular senescence as a tumor promoter

In cancer, age is an important risk factor. In aging, one of the most important mechanisms contributing to aging is the increase of senescent cells. However, the role of cellular senescence in cancer is a controversial topic, as numerous studies show that cellular senescence contributes to tumor promotion (Wiley, 2020).

first, senescent fibroblasts stimulate the growth of preneoplastic and neoplastic epithelial cells. This stimulation is caused by the secretory phenotype of these fibroblasts, accounting for 50% growth stimulation and accelerating tumorigenesis (Krtolica et al., 2001). Furthermore, in breast epithelial cells senescent fibroblasts lose the ability to differentiate, gaining invasiveness and transforming into a malignant cell type (Coppé et al., 2008). Senescent fibroblasts have the ability to impair differentiation epithelial morphology. When the epithelial phenotype is morphologically changed an epithelial to mesenchymal transition (EMT) occurs, increasing invasiveness (Jögi et al., 2012). In these epithelial cells, the ability to migrate, proliferate, and invasive is gained via the collagen matrix (Coppé et al., 2008). The secretion of vascular endothelial growth factor (VEGF), an angiogenic factor resulting in the increase of the size and number of the blood vessel and promoting the access to growth factors, leading to malignant growth, is promoted in senescent fibroblasts (Nardella et al., 2008; Carmeliet, 2005). The promotion of the formation of blood vessels was seen in malignant epithelial cells after co-injection of senescent fibroblast (Coppé et al., 2006) Also, in endothelial cells, VEGF stimulates the degradation of the extracellular matrix, resulting in an increased migration, proliferation and tube formation (Laurent et al., 2006). Other SASP components seem to have tumor-promoting properties. For example, IL-6 is known to cause increased proliferation and activation of STAT3 through binding to the IL-6 receptors (B. Wang et al., 2020). The STAT3 pathway transcribes growth regulators including, cyclin D1 and oncogenes (B. Wang et al., 2020). This pathway also targets the mTOR complex which is known to regulate senescence via modulation of the metabolism of mitochondria (Fisher et al., 2014; Xu, 2014).

Furthermore, the SASP mechanism is known to suppress tumor growth by promoting immune clearance and blocking cell division, However, there is growing evidence that suggests SASP also has a positive promoting effect on the immune environment. A mouse model showed that senescent stromal cells have a promoting effect on tumor cells due to the creation of a tumor-permissive and an immuno-suppressant environment by the increase of

suppressive myeloid cells (Ruhland et al., 2016). The SASP-secreted cytokine IL-6 has the ability to increase the number of suppressive myeloid cells and inhibit the response of anti-tumor T-cells (Ruhland et al., 2016). The SASP complex is known to secrete a large number of proteases, which play a role in the degradation of the extracellular matrix. Resulting in a more relaxed tissue structure, in turn promoting the invasiveness of cancer cells (Lecot et al., 2016).

Moreover, The SASP components IL-6 and IL-8 are inflammatory cytokines cooperating with C/EBPβ, a transcription factor, promoting the inflammatory network. By activation of the IL-6/STAT-3 pathway, senescent mesenchymal stem cells have a stimulating effect on the migration and proliferation of breast cancer cells (Kuilman et al., 2008; Di et al., 2014). Moreover, studies that blocked the secretion of IL-6 showed an impaired effect on tumor growth (Coppé et al., 2008). In experiments with the ER-+ MCF-7 breast cancer cell line, IL-6 and IL-8 have been shown to promote their phenotype resulting in the reactivation of MCF-7 cells. This reactivation results in the migration and proliferation of MCF-7 cells (Sun et al., 2018) (Figure 2).

Another characteristic of the SASP mechanism is the secretion of extracellular vesicles (EVs). EVs are able to alter characteristics of malignant cells, including angiogenesis and cell proliferation. In senescent cells, the amount of secreted EVs is increased. The study showed that the insertion of EVs, taken from healthy cells, in MCF-7 cells resulted in an increased proliferation of cancer cells (Takasugi et al., 2017)

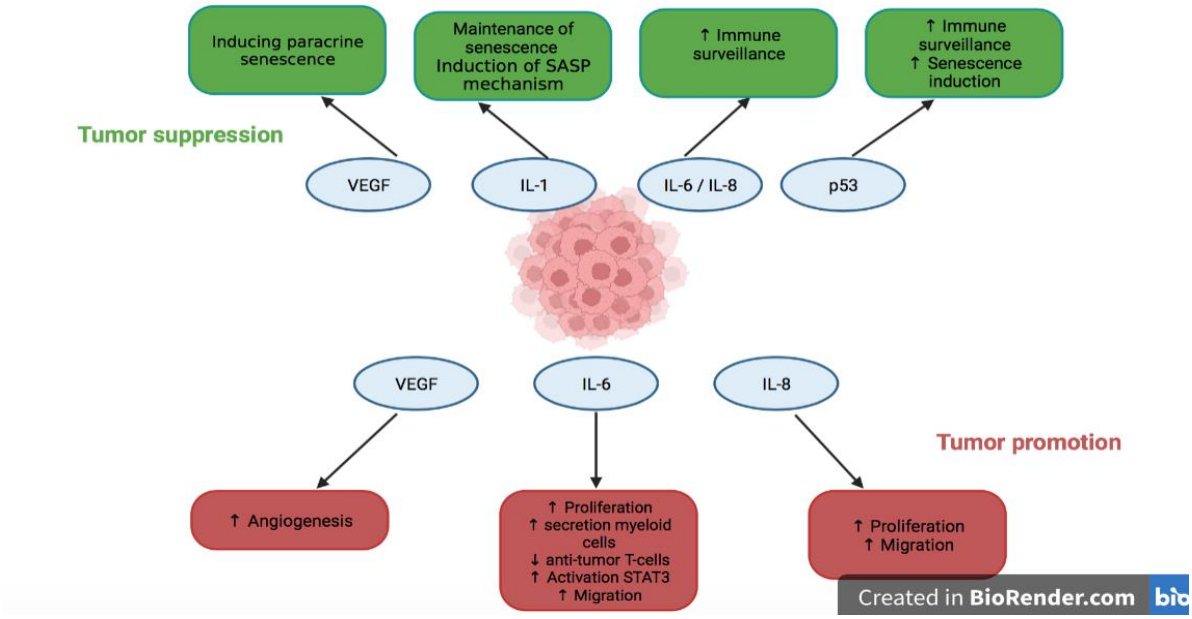

Figure 2. Different components of the SASP complex have tumor suppressing properties, such as VEGF which is known to induce paracrine senescence, IL-1 which maintains senescence and causes induction of the SASP mechanism. IL-6 and IL-8 activate immune surveillance and the tumor suppressor protein p53 also has a major role in this. However, these same cytokines, IL-6 and IL-8 have shown to increase proliferation, migration, activation

of the STAT3 pathway and secretion of myeloid cells. VEGF has an increase role in angiogenesis (figure created with BioRender).

Discussion

These characteristics of senescent cells and SASP have shown to have both beneficial and detrimental consequences in the fight against cancer. While some characteristics offer a promising way to treat cancer for example, SASP is known to secrete the inflammatory cytokines IL-6 and IL-8, studies showed that these cytokines play an important role in the induction of paracrine senescence, contributing to the suppression of tumor growth (Acosta et al., 2008). The detrimental consequences cause consequential concern in the fight against cancer. For example, these cytokines, IL-6 and IL-8 are also known to play a role in the promotion of tumor growth. These cytokines cooperate with the STAT pathway, causing an activation of the IL-6/STAT pathway. This activation results in the stimulation of the proliferation and migration of breast cancer cells (Kuilman et al., 2008).

A new drug line is recently researched showing that cellular senescence can be used as a cancer therapy. These drugs are called senolytics, as senescent cells exhibit the DDR but are able to remain alive due to avoidance of apoptosis (di Micco et al., 2020). These characteristics of senescent cells represent vulnerable features of senescent cells that can be used as a target for therapy. Based on these senescent characteristics, there is a promising combination identified containing two senolytic drugs,: dasatinib and quercetin (D + Q) (Zhu et al., 2015). This combination led to the reactivation of suppressed apoptotic signals in cells that were in a senescent state (Zhu et al., 2015). In chemotherapy DNA damage is induced resulting in the death of a number of cancer cells and inducing senescence and activating SASP in other cancer cells, called therapy-induced senescence (TIS) Takasugi et al., 2022). As previously noted, SASP is known to secrete factors causing the promotion and relapse of cancer cells. In order to prevent this promotion and relapse the use of senolytic drugs in combination with chemotherapy results in the effective targeting of TIS cancer cells resulting in the prevention of the SASP-induced relapse (Takasugi et al., 2022).

Furthermore, CDK4/6 inhibitors are observed in preclinical studies. CDk4/6 inhibitors also are known as a potent treatment in HER2-negative breast cancer (Watt et al., 2022). Abemaciclib, ribociclib or palbociclib are CDK4/6 inhibitors that are approved as a treatment for this type of breast cancer (Fassl et al., 2020). In addition to that, these inhibitors are now used in over 300 trials to treat over 30 different types of cancer (Fassl et al., 2020). These CDK4/6 inhibitors cause cell cycle arrest, inducing senescence in breast cancer cells (Watt et al., 2022). When a specific type of cancer shows resistance to these inhibitors a combination of 2 drugs has shown to be effective against this resistance, for example, triple-negative breast

cancer (Fassl et al., 2020). A characteristic of senescent cells is the increase in lysosomal mass (Lee et al., 2006). Compounds that target these lysosomes are called lysosomotropic agents. In this case, when the two drugs are combined, the resistance to the CDK4/6 inhibitor is rendered, resulting in a sensitive tumor cell (Fassl et al., 2020).

The controversial role of cellular senescence in the treatment of cancer is still not clearly understood. However, as these new drug lines are evolving, the principle of cellular senescence seems to be a potent anticancer mechanism, despite the tumor-promoting properties that are characterized in senescent cells.

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