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Review Article

An Overview of the Role of Senescence in Cancer

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Abstract

Senescence is generally viewed as a mechanism to protect humans against the development of diseases including cancer in addition to its wider physiological functions. Through this mechanism, normal cells suffering from stress are converted into senescent cells which remain metabolically active but lose their proliferative capacity. However, the persistent presence of the senescent cells can lead to unwanted consequences in that they facilitate the onset of aging and enhance the development of cancer. This narrative review examines the role of senescence in cancer and the possibility of employing senotherapies for harnessing the effects of, or eliminating, senescent cells.

***Keywords*:** Senescence, Senescent cells, Senescence and cancer, Senotherapies.

لمحة عامة عن دور الشيخوخة في السرطان

الخلاصة

يُنظر الى الشيخوخة وايقاف نمو وتكاثر الخلايا المارقة عمومًا على أنها آليه لحماية البشر من تطور الامراض بما في ذلك السرطان. من خلال هذه الآلية، يتم تحويل الخلايا الطبيعية التي تعاني من الاجهاد الى خلايا شيخوخة تظل نشطة في التمثيل الغذائي ولكنها تفقد قدرتها على التكاثر. مع ذلك، فان الوجود المستمر للخلايا المجهدة الشائخة يمكن ان يؤدي الى عواقب غير مرغوب فيها من حيث انها تعزز من تطور السرطان .تبحث هذا المراجعة السردية عن دور الشيخوخة في السرطان وامكانية استخدام علاجات الشيخوخة للسيطرة على آثار الخلايا الشائخة او القضاء عليها.

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INTRODUCTION

Our cells are continuously exposed to internal and external stresses that can damage the DNA and have the potential to lead to cancer. Several mechanisms exist to repair consequential damage resulting from exposure to those stresses. However, the persistence of stresses may require further defense mechanisms against the development of uncontrolled cell proliferation [1]. One such mechanism is senescence which is when the cells enter a stable, non-proliferative yet metabolically active state. The current view of cell senescence is that it is a highly dynamic, multi-step, and essentially irreversible entry into a non-proliferative state, although cells could be forced to re-enter the cell cycle under certain biological and epigenetic manipulation [1,2]. Senescence occurs throughout life and evolved to have a beneficial role in a variety of physiological processes including the development of the embryo, wound healing, immunity, and cancer suppression [3]. The entry of cells into a stable arrest of division represents a defense mechanism against cancer. However, cellular senescence also possesses a detrimental effect on the organism manifesting in the form of aging and age-related diseases, and in that way it can be considered a mechanism with a double-edged sword [4-9].

Senescence was first demonstrated in 1961 by Hayflick and Moorhead [10,11] who showed that dividing cells do not replicate for an indefinite time. Instead, there is a limit, referred to as the Hayflick limit, to the number of times cells can go on dividing. Human diploid fibroblast cells, for instance, are thought to be able to divide to a limit of 50±10 divisions. The restricted number of cell divisions turned out later to be due to the shortening of the telomeres. It is now recognized that telomere attrition is only one way of inducing senescence. Cells can undergo senescence in response to a myriad of triggers including oncogenic activation, mitogenic signals, oxidative damages, radiation and chemotherapeutic genotoxic stresses, epigenetic alterations, cell-organelles dysfunction, chromatin disorganization and nutrient depletion as illustrated in Figure 1 [12-17]. Different stresses can give rise to different forms of senescence for example "replicative senescence" is the type of cell arrest obtained following telomere shortening [1].

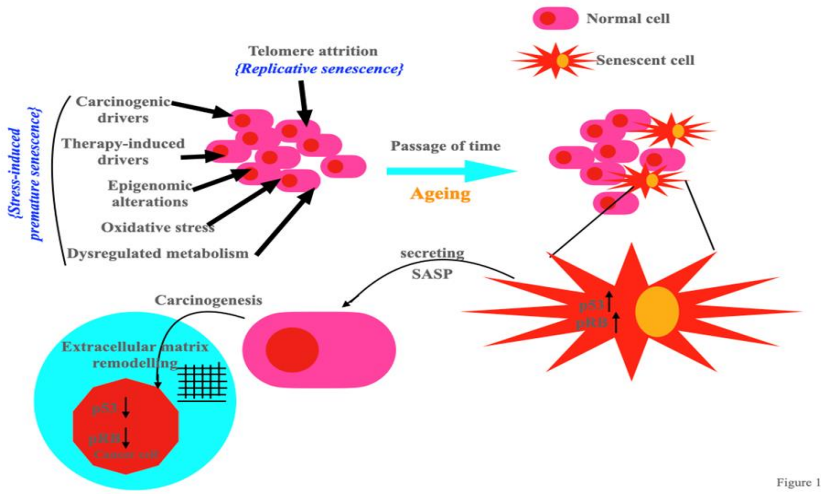

Figure 1: Senescence and cancer.

Drivers of Senescence

About six decades ago, the progressive telomere shortening was found to cause cells to enter into a stable non-replicative state which was immediately linked to the protective effect of that state on cancers [11]. In the ensuing decades, more complex views of this non-replicative state, which we now call senescence, have emerged [2]. Other cell states characterized by proliferation-arrest such as quiescence and terminal differentiation also exist but are driven by different signaling pathways. In the case of quiescence, the cells can resume proliferation in response to appropriate signals and it is essentially a reversible process [18,19]. Senescence is driven and maintained by several factors falling into two classes. The first class includes telomere shortening being the causative factor behind what is now called "telomere-dependent replicative senescence". Other drivers of senescence are generally included in the second class termed "stress-induced premature senescence" (see Figures 1, 2, and 3) [18,20].

Telomere attrition and replicative senescence

The ends of the chromosomes are called telomeres and have an imperative function in maintaining the integrity and stabilization of the DNA [21]. The polymerase enzymes that copy the DNA in preparation for cell division, are unable to completely replicate these ends of the chromosomes. Therefore, the telomeres are continuously shortening with each cell division [2,22] triggering DNA repair machinery to incorrectly recognize chromosomal attrition as a double-strand break. The telomeres lose between 50 base pairs to 200 base pairs of DNA after each S phase of the cell cycle [23]. However, human telomeres could be up to 15,000 base pairs long, and therefore many cell divisions are possible before they become critically short and dysfunctional [24]. It could take only one such dysfunctional telomere to trigger senescence [25,26]. Repairing such an assumed break, and fusing chromosomes due to the absence of the normal protective caps, drive the cells into rampant genomic instability which is a major risk factor for cancer. Most of our somatic cells lack the expression of the telomerase, the reverse transcriptase enzyme that replenishes the lost DNA from the telomeres, and hence are in danger of becoming cancerous through the acquisition of dysfunctional telomeres. This telomere shortening elicits an inherent cell DNA repair program DNA damage response (DDR) [27-30]. Telomeres are notoriously difficult to repair, and it has been suggested they are effectively unrepairable, causing a persistent DDR. The DNA damage response mechanism will then arrest cell proliferation, and maintain the senescence state primarily through the activity of the tumor-suppressor proteins P53 (also known as TP53-tumor protein 53) and pRB (retinoblastoma protein), which is an outcome intended as an anticancer mechanism [29,31-33]. Proliferative

arrest resulting from telomere shortening is usually given the name telomere-dependent "replicative senescence" to distinguish it from stress-induced "premature senescence" which encompasses the other forms of senescence [18,20].

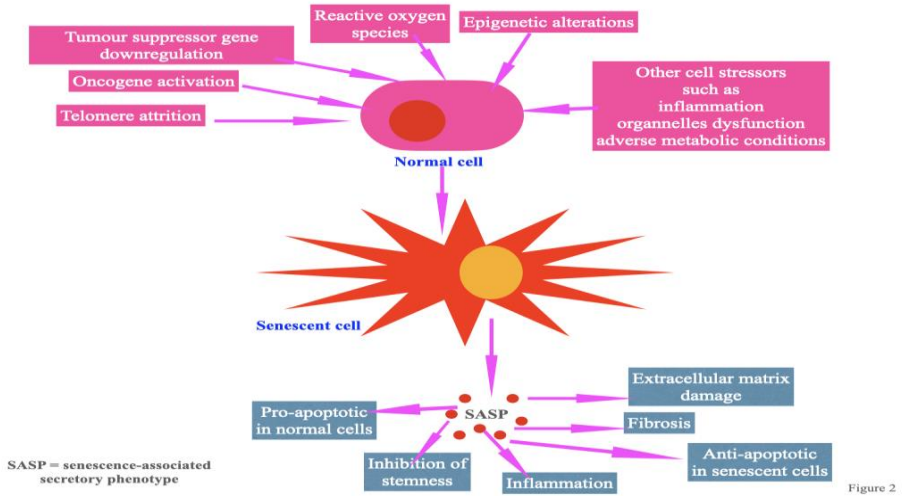

Figure 2: Drivers of senescence.

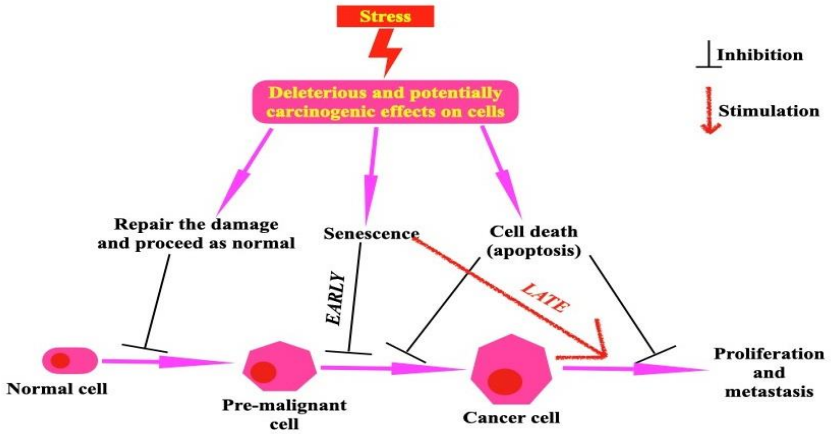

Figure 3: Early and late phases of senescence.

Stress-induced premature senescence

Several years after the first description of replicative senescence, other cellular stresses such as the *in vitro* expression of activated oncogene were found to produce an indistinguishable phenotype called "premature senescence" that is independent of telomere attrition [34-36]. Following that, several other physiological changes are also reported to induce premature senescence. These changes can be triggered by intrinsic stressors such as activation of oncogenes, downregulation of tumor suppressor genes, oxidative damage, cell-organelles dysfunction, and chromatin disorganization or by extrinsic stressors such as UV radiations and chemotherapy treatments. Premature senescence is likely to be the most important inducer of cellular senescence since many cell types never exhaust their maximum replicative potential during the life span of the organism and thus do not enter replicative senescence [37]. The activation of oncogenes and the inactivation of tumor suppressor genes are often grouped under one antiproliferative response termed oncogene-induced senescence (OIS) [36,38,39]. Details of the full list of premature senescence inducers are beyond the scope of this overview and only important stressors concerning cancer are discussed briefly below. The activation of oncogenes, such as *HRAS* (Harvey rat sarcoma) and *MYC* (myelocytomatosis), triggers oncogene-induced senescence in normal cells [34,40]. This appears, at first glance, paradoxical since oncogenic proteins are drivers of carcinogenesis. However, the sole activation of the *HRAS* gene, for example, is not sufficient to drive transformation and requires the cooperation of other drivers [41,42]. The expression level of the oncogene appears to be important for RAS-induced senescence as it occurs only when RAS is overexpressed [43,44]. Overexpression of *HRAS* in the absence of additional hits drives cells into senescence. It is thought that oncogenes induce senescence as a failsafe program to counteract excessive mitogenic stimulation that might allow the proliferation of abnormal cells [24,44]. This thought is supported by the finding that mouse cells when cultured

in a serum-free medium (lacking mitogens) are seen to resist RAS-induced senescence [45]. Moreover, rodent cells lose the ability to senesce in a serum-free medium suggesting that excessive mitogenic stimulation is needed for their senescence program [24,46,47]. The relevance of OIS is demonstrated in benign naive (moles) in humans. These moles contain cells that express the oncogenic form of BRAF protein and are senescent and may remain dormant for decades suggesting that OIS plays a role in the suppression of carcinogenesis. For moles to develop into cancer, they require additional mutations, notably in *TP53* or *p16* genes that prevent or reverse the senescence state [48-50]. Loss of tumor suppressor genes, such as *PTEN* (phosphatase and tensin), can also induce senescence [49]. Proliferation arrest during senescence is mainly established and maintained through the canonical pathways involving p53 and pRB proteins [51-53]. The chronic activation or overexpression of p53 or pRB is generally sufficient to induce senescence. Radiotherapy and chemotherapy can cause severe DNA damage, which is considered to be the main cause of senescence induction, in their target cells as well as surrounding cells [1,54-57]. Other possible explanations underlying cellular senescence following chemo-radiotherapy are the production of ROS (reactive oxygen species) or the inhibition of any remaining telomerase activity [58,59]. Tumor mass often exhibits a mixed phenotype of cells following chemotherapy with some showing senescence and others showing apoptosis [60]. Tumor cells are more likely to senesce in response to chemotherapy if they contain the wild-type *p53* gene [61,62]. Therefore, the status of the *p53* gene in the tumor could have a practical significance for the success of chemotherapy. It was found that low doses of chemotherapeutic agents can trigger senescence in human cancer cells while higher doses can induce apoptosis [61,63,64]. Chemotherapeutic agents that are known to induce senescence include Docetaxel, Bleomycin, Cyclophosphamide, Doxorubicin, Vincristine, Etoposide, and Cisplatin [65]. Saleh et al. provided a comprehensive review of both conventional and targeted therapeutics that have been shown to induce senescence [66]. Radiotherapy can also activate pathways leading to senescence or apoptosis, however, as radiotherapy is used locally this treatment causes less collateral damage to normal tissue and potentially fewer secondary cancers [67]. In humans 90% of the oxygen is consumed by the mitochondria and up to 5% of that is converted to superoxide and eventually to reactive oxygen species (ROS) [68,69]. Reactive oxygen species are involved in a variety of functions from preparing for childbirth and the defense of the organism to the regulation of a variety of cellular functions such as proliferation, differentiation, senescence, and apoptosis [70]. Therefore, a reasonable level of oxidative stress is beneficial. However, excessive ROS production may affect the nucleic acids and nutrients causing major damage to cells containing them. Cells experience oxidative stress when they excessively produce ROS. The latter participate in several intracellular reactions leading to the accumulation of oxidative damage in molecules and organelles [69]. Oxidative stress can also cause DNA damage leading to the activation of DDR and the consequent proliferation arrest [29,56,71,72]. The chromatin state of the histones around the DNA determines the extent to which genes are active (euchromatin state) or silent (heterochromatin state). These different chromatin states are driven mainly by the methylation or acetylation status of the histones. Deacetylation of histones by histone deacetylases (HDACs) tightens their interaction with the DNA resulting in a closed chromatin configuration, heterochromatin, and the inhibition of the relevant gene expression. Senescent cells show widespread perturbations in their epigenome manifesting in the formation of heterochromatin which represses several proliferative genes [2,73]. Senescence is also observed using histone deacetylase inhibitors [73]. The finding that HDAC inhibitors also caused senescence appears to conflict with the role of heterochromatin in establishing and maintaining senescence as these compounds promote euchromatin formation [73]. It is not known how senescence can be triggered by both heterochromatin and euchromatin formations [74]. Both manipulations may cause changes in chromatin organization that may alter the expression of a different set of critical genes and the response may be cell type-specific. Understanding this paradox could be important as HDAC inhibitors hold the promise for treating certain cancers [75].

The Senescent Phenotype

The stresses, mentioned above, induce the cells to acquire a phenotype that is different from their normal state and are often, but not always, resistant to cell death [76]. Senescent cells are characterized by having an enlarged structure and flattened smoothed shape when compared with their proliferating counterparts. However, these features and other hallmarks of senescent cells are often shared with other cellular states such as quiescence and terminal differentiation [77]. The commonly held features of senescent cells are now brought together under a set of four characteristics; a) cell-cycle withdrawal, b) macromolecular damage, c) deregulated metabolism and d) secretion of several chemicals collectively known as SASP (senescence-associated secretory phenotype) [18,77]. These four hallmarks are the result of striking changes in gene expressions, brought about by genetic and epigenetic alterations, and some of these alterations might serve as surrogate markers of senescence. Senescence is a highly heterogeneous phenomenon and some of its features may vary according to the trigger and cellular context. There is, as yet, no single characteristic that can robustly identify senescent cells. Identification of senescent cells requires the use of a combination of features [39]. It is recommended that three different markers, within the same cells, are used for the detection of senescent cells [78]. These three different markers may include: a) a

cell-cycle marker, b) increase lysosomal mass and content and c) a relevant feature from the cell nucleus.

Cell-cycle withdrawal

Cell-cycle arrest, while remaining metabolically active, is a crucial characteristic for the identification of all types of senescence despite it not being a unique marker [42,79,80]. Multiple cellular mechanisms, apart from senescence, can drive a stable replicative arrest. However, the inability to express genes required for proliferation, even in the presence of pro-mitogenic signals allows senescence to be distinguished from quiescence, which is another non-proliferative cell state that is readily reversed by mitogens [81,82]. One of the characteristics of senescence is the overexpression or activation of the cell cycle inhibitor proteins INK4A (p16) and ARF (p14) encoded by the *CDKN2A* gene, p21 encoded by the *CDKN1A* gene and p53 encoded by the *TP53* gene [1]. The upregulation of these cell cycle inhibitors commonly retards cell proliferation leading to senescence [77]. The augmented levels of cell-cycle inhibitors are used as senescence biomarkers [42]. Cell cycle withdrawal is also associated with wide epigenetic alterations [83,84]. Senescence-associated heterochromatin foci (SAHF) appear to different extents in senescent cells depending on the particular stimulus driving the process [85].

Macromolecular damage

The substantial accumulation of damaged DNA, protein, and lipid damage is another characteristic feature of senescence. The progressive attrition of the DNA at the telomeres was the first molecular feature associated with senescence which culminates in the activation of DDR and cell-cycle arrest [18,31]. Although about half of the persistent DNA damage in senescent cells can be traced back to the telomeres, other stressful insults can trigger senescence by inducing irreparable DNA damage. Such insults can include genotoxic agents (ionizing and UV radiations, chemotherapeutic agents, and ROS) [77]. As senescent cells remain metabolically active, ROS accumulates and contributes further to oxidative DNA damage at the telomeric Guanine (G)-rich repeats. This, in turn, facilitates the assembly of the DNA repair machinery involved in the DDR system. The detection of these modifications is widely used to identify senescence [86,87]. Protein damage is another hallmark of senescence and a prominent cause of such damage is ROS [88]. Protein oxidation can be irreversible and the oxidative products are often eliminated by the ubiquitin-proteasome system (UPS) or autophagy. As UPS and autophagy are active processes in senescence cells their activities could prove useful in characterizing the senescence state [18]. Protein phosphatases damaged by ROS and their subsequent removal by the proteasome-dependent protein degradation system leads to the hyperactivation of ERK (extracellular signal-regulated kinases) signaling triggering senescence [89-91]. The accumulation of damaged proteins also increases ER (endoplasmic reticulum) stress triggering the unfolded protein response UPS and senescence. Senescent cells also exhibit upregulation of anti-apoptotic response aimed at counteracting the impact of DNA and protein damage [92]. Lipids are essential nutrients for energy production, cell membrane structure, and signal transduction. Senescent cells exhibit altered fat metabolism although it is unclear how this contributes to the senescence phenotype. Senescent cells often harbor dysfunctional mitochondria which can induce ROS-driven lipid damage [18].

Deregulated metabolism

Senescent cells rely on mitochondrial metabolism and glycolysis to remain metabolically active and obtain their demand for energy in the form of ATP (Adenosine triphosphate) [93]. The ability of the mitochondria to produce ATP is compromised during senescence [94]. Instead, the mitochondria release more ROS leading to enhanced protein and lipid damage as well as telomere attrition and DDR activation [89]. The altered ratio of the less phosphorylated Adenosine to ATP contributes to cell-cycle withdrawal through the activation of AMPK (Adenosine monophosphate-activated protein kinase) signaling [94]. Mitochondrial function is also implicated in SASP regulation. Mitophagy (a form of mitochondrial autophagy) in senescent cells appears to suppress SASP [95]. However, as the dysfunction of the mitochondria appears in other cellular processes, it is not a consistent biomarker of senescence. The lysosomes represent the last degradation compartment for several cellular processes including phagocytosis, endocytosis, and autophagy where the materials are broken down and recycled. Lysosomes in senescent cells increase in number and size giving rise to the granular appearance of the cytoplasm [96]. The increase in lysosomal number does not necessarily imply an increase in activity as it could reflect an attempt by the cell to re-address the balance in number caused by the accumulation of dysfunctional lysosomes. The increased lysosomal mass has been linked to a rise in β-galactosidase activity [97]. However, although senescence-associated β-galactosidase (SA- β-gal) is prominent in senescent cells, it is neither essential nor a determinant of senescence phenotype [97]. Nevertheless, an elevated number of lysosomes showing an enhanced lysosomal β-galactosidase activity are the most widely employed marker for the detection of senescence.

Secretion of "SASP" factors

Senescent cells secrete a collection of substances termed senescence-associated secretory phenotype (SASP). This collection of substances is composed of diverse pro-inflammatory cytokines, chemokines, growth factors, angiogenic factors, and proteases. The SASP factors play a key role in reinforcing and propagating the senescence phenotype. These factors maintain the senescence state in a cell-autonomous (autocrine) fashion and propagate that state to other cells through a cell-nonautonomous

(paracrine) manner [4,98-100]. The SASP can activate immune responses that eliminate senescent cells [101,102]. The SASP mediates developmental senescence, wound healing, and tissue plasticity and contributes to a persistent tissue inflammation known as inflammaging [18]. Furthermore, the SASP can recruit immune suppressive cells, drive angiogenesis and metastasis and thus aid the process of carcinogenesis [4, 103,104]. The paracrine effects of SASP can have a detrimental influence depending on the nature of the stress that triggered senescence, the cell types involved and the length of time since senescence first started [105]. Furthermore, the SASP composition and strength vary substantially depending on the duration of senescence, the nature of the stimulus, and the cell type [106].

Two Phases of Senescence in Carcinogenesis

The loss of senescence response appears to be crucial as a cause of cancer development [24]. Cells of genetically-engineered mice that are deficient in genes essential for senescence, such as the *p53*, fail to senesce in response to appropriate stimuli and the animals are invariably cancer-prone [48,49,107]. Furthermore, humans with Li-Fraumeni syndrome, a hereditary genetic condition caused mainly by mutations in *p53* or *CHK2* (checkpoint kinase 2) essential senescence genes, are also more susceptible to developing cancers [108,109]. However, failure of senescence is usually insufficient alone for malignant transformation. For example, the use of telomerase does not, ordinarily, confer malignant properties in normal cells yet this enzyme prevents senescence by repairing the telomere attrition. Such cells remain in a proliferative, non-malignant, state until they acquire further oncogenic mutations activating oncogenes or inactivating tumor-suppressor genes [49,107,110,111]. The role of senescence in carcinogenesis truly illustrates a two-sided function of this phenomenon. While senescence in tumor cells can impede the proliferation of these unconstrained cells, senescent non-tumor cells have the potential to promote cancer [112]. Studies have shown that in neoplastic tissues where oncogenic activation and senescence markers have been identified, there is a tendency to develop into malignant tumors [49,113]. Senescence appears to suppress tumor growth in the early stages while contributing to tumor development in later stages. With aging, this phenomenon becomes more pronounced as senescent cells accumulate and supply even more pro-tumorigenic factors [114]. Humans and other multicellular eukaryotes have developed three main mechanisms to counteract the effects of potentially carcinogenic stresses. These mechanisms are; a) repairing the damage caused by the stress, b) permanently arresting the cell-cycle progression (senescence), and c) eliminating the affected cells through killing (apoptosis). As to which of these routes the cell will follow when stressed, is highly dependent on the context [92,115]. If the stress damage is unrepairable, the cell might opt to go for option (b) as the first barrier against cancer initiation [48-50,111,116]. The senescence pathway generally involves two phases, an early anti-tumor phase (see Figure 4) and a late pro-tumor phase (see Figure 5). The effects of senescence on the progression of cancer in these two phases are likely to be highly context-dependent and mediated by a complex interplay between SASP and TME (tumor microenvironment).

The anti-tumor early phase of senescence

Senescence initiation in cancer cells is mainly oncogene-induced (OIS) or therapy-induced (TIS). Oncogene-induced senescence generally occurs in the early phase limiting proliferation and maintaining cancer cells in a non-invasive state [36,113]. Both OIS and TIS cells secrete interleukin IL1α, a crucial SASP initiator and regulator [117].

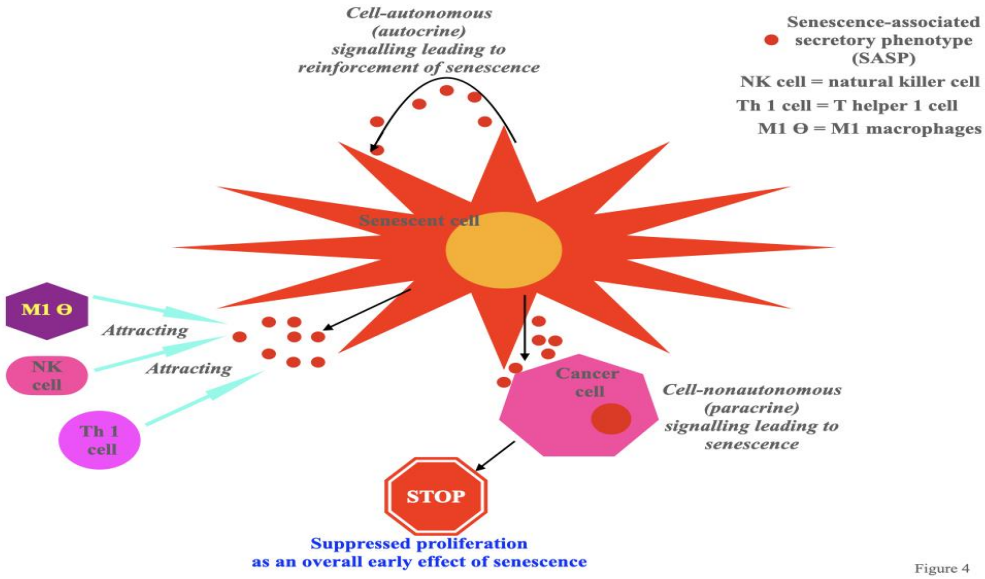

Figure 4: Early effects of senescence.

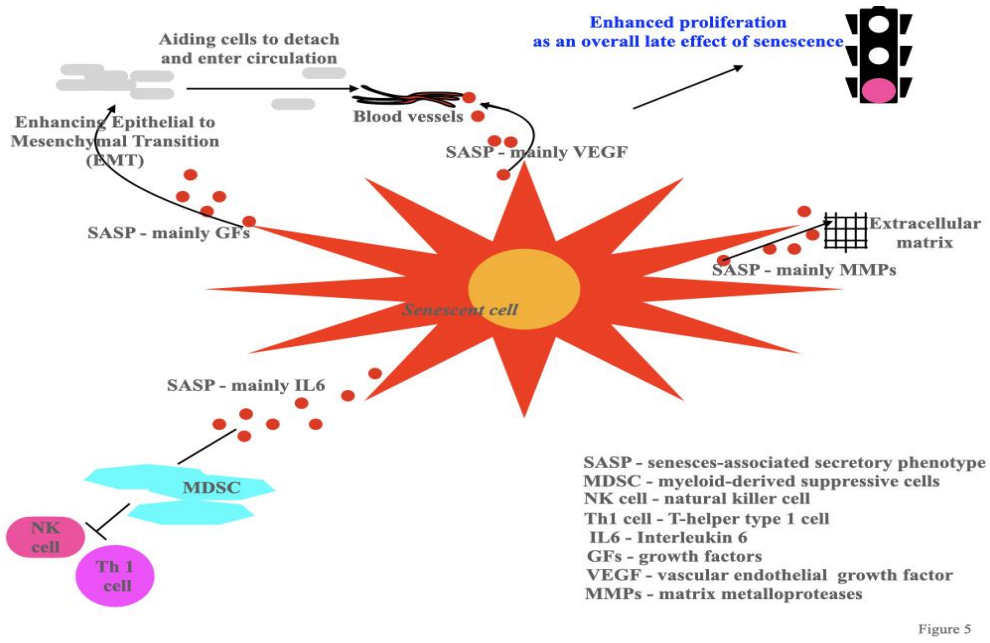

Figure 5: Late effects of senescence.

The IL1α triggers an autocrine inflammatory response through the activation of NF-kB leading to the transcription of IL6 and IL8 [117]. These latter interleukins will reinforce senescence through the increased production of ROS and sustained DDR [98,117]. IL1α also mediates paracrine senescence to suppress cancer progression [100]. IL1α, IL6, and IL8 also mediate the recruitment of M1 macrophages, NK (natural killer) cells, and Th1 (T-helper 1) cells to TME. These infiltrating immune cells drive the elimination of senescent cancer cells, and possibly non-senescent cancer cells although this is not yet proven [118,119]. This early phase defines senescence as a physiological tumor-suppressive process and was illustrated by melanocytes possessing mutation V600E in their *BRAF* oncogene [120,121]. These melanocytes show enhanced proliferation initially in the form of moles, followed by cell-cycle arrest and the display of other characteristics of senescence which prevent the moles from developing into skin cancer. Moreover, some pre-cancerous lesions contain a large number of cells that express senescence markers suggesting that this process plays a role in halting the progression to full malignancy [24]. This highlights the initial role of senescence in cell-cycle arrest as a tumor-suppressive mechanism. Further tumor-suppressive action of senescence is illustrated by the phenomenon of tumor reversion. Investigations have found that the re-establishment of P53 expression in mouse models of breast and liver cancers induces senescence leading to cancer regression showing that senescence, in this context, not only prevents the acquisition of malignancy but also aids in the regression of established tumors [122,123]. The senescence pathway will also lead to the modulation of the microenvironment by senescent cells as illustrated in Figure 4. The SASP components, a classic hallmark of senescence, reinforce senescence and promote immune surveillance in the early phase thereby enhancing the removal of premalignant cells [77,105,118,119,123]. The reinforcement of senescence occurs in both cell-autonomous (autocrine) and cell-nonautonomous mechanisms thereby strengthening the tumor-suppressive effects [98,100,124]. Senescent cells, via SASP, can induce paracrine cell-cycle arrest in neighboring cells thus acting as a barrier against tumor growth. The SASP can activate the immune surveillance machinery to clear senescent and proliferating tumor cells [125].

The pro-tumor late phase of senescence

The first study that illustrated the pro-tumor aspect of senescence was when human fibroblasts undergoing replicative senescence were able to promote the growth of co-cultured epithelial cancer cells and able to form tumors in mice when co-injected. This paradoxical effect, resides in the way the SASP factors can manipulate the environment, a phenomenon known as maladaptive senescence [4,105,124,126-129]. This can be seen in cancer patients in what is termed therapy-induced senescence (TIS) although this term is not limited to cancer cells and can occur in non-cancer cells [42,130]. Therapy-induced senescence can initially be beneficial in blocking tumor progression through cell-cycle arrest. However, TIS can also impair the elimination of senescent cells through its immune-suppressive chronic effects thereby promoting aging-

related phenotypes. Therapy-induced senescence has been linked with the aggravation of the side effects of treatments as well as the relapse of cancer. This is in agreement with clinical observations showing that chemotherapy treatments can induce premature aging, particularly following high doses [131,132]. The SASP secretions support chronic inflammation within the TME which in turn can promote cancer development [133,134]. The TME consists of senescent tumors, non-senescent proliferating tumor cells, stromal cells and infiltrating immune cells. The main infiltrating immune cells are T cells, NK cells, myeloid-derived suppressive cells (MDSCs) and macrophages. The latter cells can have either anti-tumor activity (M1) or pro-tumor activity (M2). A recent study was able to show that SASP components were able to not only increase the progression of existing tumors but also aid in the initiation of cancer [135]. Although the SASP secretions promote immune surveillance and clearance of tumor cells initially, these secretions could become deleterious when the immune system is exhausted or when senescence is compromised through the selective inactivation of essential components such as p53 [77]. This will ultimately lead to escape from senescence-mediated repression of carcinogenesis and the acquisition of more malignant phenotypes. IL6 and IL8 are thought to be important mediators of the pro-tumorigenic, late-stage, effects of senescent cells because they create a chronic inflammatory environment that supports cancer development [136,137]. In addition, IL6 and IL8 also drive the transcription of genes encoding MMPs (matrix metalloproteinases) and drive EMT (epithelial-mesenchymal transition) thereby promoting cancer invasiveness [138,139]. IL6 also recruits MDSCs (myeloid-derived suppressor cells] to TME [140]. These cells block IL1α signalling and therefore antagonize the establishment of senescence in cancer cells and block immune surveillance [103,119,140]. Thus, SASP factors create an immunosuppressive environment facilitating tumor growth [140,141]. Senescence also drives EMT, which is a type of cellular transition that provides tumor cells with a more favorable milieu for cancer progression and to acquire enhanced metastatic abilities [142-145]. For example, IL6 produced by senescent mesenchymal stem cells is considered a significant driver of cancer progression and has been found to promote the growth and metastasis of breast cancer [112,146]. Metastasis can be accomplished either by promoting the migratory capability of tumor cells or through the preparation of a more suitable microenvironment in distant organs for tumor seeding. The SASP factors were detected in the blood of patients following chemotherapy treatment as well as in experimental animals engrafted with senescent tumor cells [77, 147,148]. Moreover, senescence, through SASP, also stimulates angiogenesis through increasing tumor vasculature [127]. In contrast to the early senescence-mediated immune surveillance, an age-related accumulation of $p16^{INK4A}$ –positive senescent T cells occurs which are implicated in the negative regulation of the immune response and the consequent pro-tumorigenic phase [149]. The essentially irreversible cell-cycle arrest induced by senescence has been challenged in recent years by studies pointing out that TIS in cancer cells can, albeit very rarely, be reversed to resume proliferation [62,150,151]. The ability of rare cells to re-enter the cell cycle is thought to be the cause of cancer relapse in some patients [62,152]. Milanovic *et al*. showed that Adriamycin-treated lymphoma cells exhibit an increase in the level of SA-β-gal, suggesting the induction of senescence, but later re-acquire proliferative capabilities to promote cancer relapse [153]. These emergent dividing cells were shown to possess a self-renewal capacity, similar to a stem cell, resulting in aggressive proliferation. In a breast cancer study, 15 out of 36 samples from patients treated with chemotherapy showed an increase in SA-β-gal compared to 2 out of 20 samples from patients who did not undergo chemotherapy treatment [154]. It is not yet known whether this phenomenon represents a true reversion or simply reflects an initial senescence escape in the first place. Recent research has linked senescence reversion following TIS to polyploidization, the event in which cells gain more than two sets of chromosomes [155,156]. While anti-cancer treatments may induce senescence and/or polyploidy, it has been suggested that cancer relapse is dependent on polyploidy, rather than senescence, thus re-enforcing the assumption that TIS may use polyploidization to revert to a proliferative state [152,154]. The OIS cells were also found, in another study, to be capable of re-entering the cell cycle, particularly by restoring telomerase activity [157]. Lately, it was found that the small extracellular vesicles secreted by senescent cells as part of SASP, can also promote cancer development [158,159]. Furthermore, senescent stromal cells produce a large number of small extracellular vesicles which alter the expression profile of recipient cancer cells and can enhance their aggressiveness and promote drug resistance [160].

Targeting Senescence in the Treatment of Cancer

Historically senescence has been described as a cancer-protective mechanism inhibiting the proliferation of neoplastic cells [161]. The senescent cells, although remaining metabolically active, have exited the cell cycle and thus are viewed as a desirable outcome of cancer therapy as this reduces tumor growth. However, as the senescent cells continue to perform metabolic functions and adversely influence adjacent cells with their secretory factors, they will need to be removed to minimize cancer-regression risk. As mentioned earlier, chemoradiation treatment of cancer can cause what is called therapy-induced senescence (TIS) which can have long-term adverse consequences when the senescent cells are chronically present [162]. Ideally, the senescent cells should be specifically targeted and eliminated in conjunction with, or soon after, cancer treatment. The strategies to achieve this may be classified into three categories: 1) reducing the accumulation of senescent cells using non-pharmacological interventions, 2)

employing drugs to attenuate the influence of SASP, and 3) employing drugs to reduce the number of senescent cells [163]. Compounds that suppress the detrimental effects of SASP factors are called senomorphics while those that selectively kill senescent cells are called senolytics [164].

Non-pharmacological interventions

Studies have shown that a 26% caloric restriction of mice diet for three months reduces the number of senescent cells in these animals (see Figure 6) [165]. The caloric restriction reduces the availability of reactive oxygen species (ROS) and insulin growth factor 1 (IGF1) which are considered important drivers of senescence.

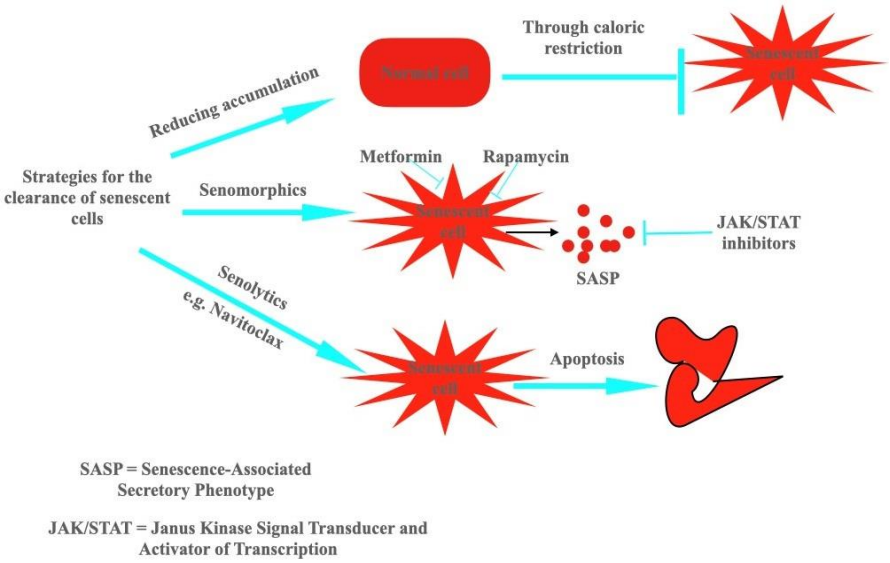

Figure 6: Strategies for the elimination of senescent cells.

Employing senomorphics

The compounds that are employed to reduce the undesirable effects of SASP factors are usually approved for other indications. Good examples of these molecules are Metformin and Rapamycin [166]. The long-term use of Metformin is associated with extended life/health span independent of its antihyperglycemic effect albeit through an unknown mechanism [167]. Rapamycin, on the other hand, inhibits the transcription of several members of the SASP factors, through binding to its signaling ligand mTOR (mammalian target of rapamycin), thereby increasing the autophagy of senescent cells [168,169]. Table 1 lists some selected examples of senomorphics.

Employing senolytics

In theory, these compounds would only need to be used when required and sporadically to eliminate any accumulated senescent cells over a while. This was, and still is, a good reason for focusing most of the efforts on developing this category of molecules [163]. Senolytics selectively kill senescent cells by targeting molecular pathways critical to the survival of these cells such as the pro-survival and anti-apoptotic mechanisms. The first senolytic therapy reported was a combination of Dasatinib and Quercetin. Dasatinib is an approved drug for cancer treatment and Quercetin is a naturally occurring flavonoid. This combination acts as a senolytic by targeting the anti-apoptotic pathway crucial for the existence of senescent cells [164]. The Dasatinib/Quercetin combination has been tested in several human clinical trials for the treatment of conditions such as idiopathic pulmonary fibrosis, chronic kidney disease, and Alzheimer's disease [164]. Navitoclax is another important senolytic and is a member of Bcl2 inhibitors (Bcl2 being important proteins regulating cell survival and resistance to apoptosis). The Bcl2 inhibitors share a common side effect, that of thrombocytopenia, which could limit their clinical use. Selected examples of senolytics are given in Table 1. The immune surveillance system could be employed to eliminate senescent cells as well as target their SASP factors [44]. This can involve the enhancement of the natural ability of the immune system to clear senescent cells which have acquired altered expression profiles compared to normal cells. The autologous transplant of immune cells after being challenged *ex vivo* with antigens specific to the senescent cells and the use of antibodies that target senescent cells

for removal by natural killer cells are just two examples [163,170].

Table 1: Examples of senotherapeutics

compound (s)	Mechanism of action	References
SENOLYTICS		
Quercetin and Dasatinib	Inhibition of various anti-apoptotic pathways	175,201
Navitoclax	Inhibition of BCL2 family of anti-apoptotic proteins	174
Alvespimycin, Geldanomycin, Tanespimycin	HSP90 inhibition	173,177,199
FOXO4-DRI	Disruption of FOXO4-p53 interactions	176,200
UBX0101	Inhibition of MDM2	178
P5091, P22077	USP7 inhibition	179,180
Fisetin	Naturally occurring flavonoid promoting apoptosis via anti-apoptotic pathways	181
GL-V9	Synthetic flavonoid promoting apoptosis via anti-apoptotic pathways	182
Cardiac glycosides such as Ouabain and Digoxin	Promoting apoptosis via anti-apoptotic pathways	183,184
Gemcitabine, Duocarmycin, Nav-Gal	Galactose-modified prodrugs are produced by linking a cytotoxic drug to a galactose derivative.	185,186,187
PZ15227	A PROTAC-type product produced by linking Navitoclax to Pomalidomide	188
Fenofibrate	Agonist of PPARα	172
Azithromycin	Possibly through induction of glycolysis and autophagy	189
SENOMORPHICS		
Rapamycin	mTOR inhibitor suppresses SASP	190
Metformin	suppresses SASP	166,191
Resveratrol	SIRT1 activator senomorphic at low concentration	192
Aspirin	suppresses SASP	193
SR12343	Inhibition of IKK/NFkB	194
SB203580	Inhibition of p38MAPK	195
Ruxolitinib	Inhibition of JAK1/2	171
KU55933	Inhibition of ATM	196
Simvastatin, Atorvastatin, Pravastatin	Inhibition of HMG-CoA reductase	197,198

BCL2 - B-cell lymphoma 2; HSP90 - heat shock protein 90; FOXO4 - forkhead box protein O4; MDM2 - murine double minute 2 (an E3 ligase); USP7 - ubiquitin-specific protein 7; PROTAC - proteolysis targeting chimeras; PPARα - peroxisome proliferator-activated receptor alpha; mTOR - mechanistic or mammalian target of rapamycin; SASP - senescence-associated secretory phenotype; SIRT1 - Silencing information regulator 2-related enzyme 1; IKK/NFkB - IkappaB kinase/nuclear factor kappa-light-chain-enhancer of activated B cells; p38MAPK - mitogen-activated protein kinase; JAK/12 - Janus kinase 1/2; ATM - ataxia telangiectasia mutated; HMG-CoA - 3-hydroxy-3-methylglutaryl coenzyme A

Concluding Remarks

Senescence is considered an evolutionarily conserved mechanism acting against the development of cancer through the removal of rogue cells from the proliferative potential. However, the accumulation of senescent cells has the unwanted outcome of causing aging and can facilitate the development of cancer hence their identification and removal are required. Much work is still needed to characterize, and discover biomarkers specific to, senescent cells. Clinical trials employing senotherapies in the field of cancer treatment are ongoing but remain distant from clinical application. Regarding deployment of senescence in cancer treatment, an emerging strategy is the use of a combination of anti-cancer drugs given sequentially to target different vulnerabilities and prevent the acquisition of therapy resistance. In this context, a senescence-inducing anti-cancer drug is given first to halt the advancement of neoplasia followed by a senolytic agent for the targeted elimination of senescent cells.

Conflict of interests

The author declares no conflict of interests.

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