The Pharmaceutical and Chemical Journal, **2023, 10(1):14-20**

Available online www.tpcj.org

Review Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

A Review on Cellular Senescence: Mechanism and Therapeutic Potential

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Abstract Cellular senescence is a stable cell cycle arrest that can be triggered in normal cells in response to endogenous and exogenous stresses, including telomere dysfunction, oncogene activation and persistent DNA damage. Therefore, the identification, characterization, and pharmacological elimination of senescent cells have gained attention in the field of aging research. Cellular senescence occurs in response to Senescent cell extrinsic activities related to the activation of a senescence-associated secretory phenotype, amplify the impact of cell-intrinsic proliferative arrest and contribute to impaired tissue regeneration, chronic age-associated diseases and organismal ageing. This Review discusses the mechanisms and modulators of cellular senescence establishment and induction of a senescence-associated secretory phenotype, and provides an overview of cellular senescence as an emerging opportunity to intervene through senolytic and senomorphic therapies in ageing and ageing-associated diseases. Here, we describe the molecular marker of senescence phenotypes and they are used for identifying senescent cells *in vitro* and *in vivo*. We also highlight the importance that these levels of regulations have in the development of therapeutic targets.

Keywords Senescence, Cellular senescence, Ageing, Replicative senescence, Senescence Associated secretory phenotype (SASP)

Introduction

Senescence derives from 'senex', a Latin word meaning old man or old age, Cellular senescence is a process in which cells enter into irreversible cell cycle arrest and that occurs due to excessive stress or damage to intracellular or extracellular components of the cell [1]. To avoid malignant transformation after the stressor`s activity, cellular senescence refers to the arrest in the G1 phase of the cell cycle [2]. The cell loses its ability to perform the basic metabolism and participate in tissue homeostasis. The cell undergoes some crucial changes which cause phenotypic changes like resistance to programmed cell death, abnormality in morphology, altered gene expression, and a complex senescence-associated secretory phenotype (SASP) [3-4].

For the first time, in 1961 this phenomenon was described by Hayflick in human fibroblasts serially passaged in culture. In which they found that normal human fetal fibroblasts in culture reach a maximum of approximately 50 cell population doublings before becoming senescent [5]. Telomere shortening is considered the most prominent inducer of cellular senescence. After each round of DNA replication, the end part of chromosomes, also called the telomeres, gets shortened due to poor telomere maintenance. This shrinkage of telomere after a limit can lead to damage to the DNA structure and induce a DDR like the DNA DSBs [6-7]. The limited proliferative capacity of human fibroblasts in culture condition is called "replicative cellular senescence".

This process was later associated with telomere shortening or dysfunction which triggers DNA damage response and subsequent cell cycle arrest. However, senescence can also be induced in response to other stress conditions such as

oncogene activation, oxidative stress, or metabolic stress before any telomere shortening. This is defined as "premature cellular senescence".

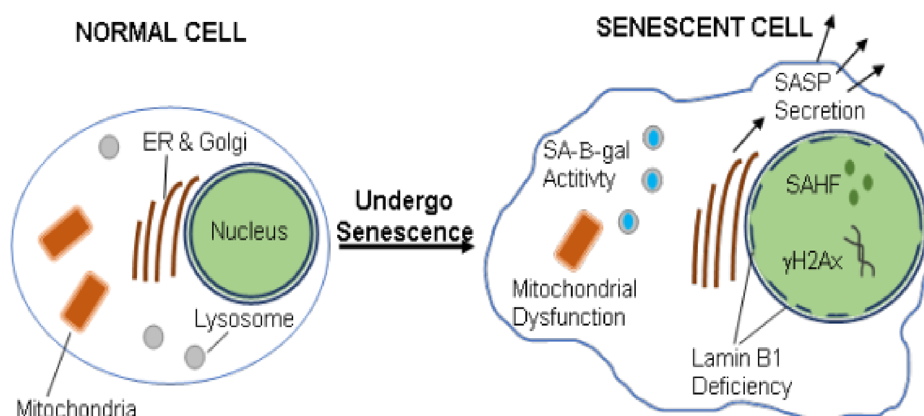

Figure 1: Cellular changes during senescence- Recognizable features of senescent cells include SA-β-gal, chromatin changes (SAHF), nuclear lamina and envelope defects (Lamin B1 deficiency), SASP secretion, mitochondrial damage, DNA Damage markers (γH2Ax) with an overall flattened and enlarged morphology in cultured cell

Characteristics of Senescence:

Senescent cells are associated with many age-related degenerative phenotypes, both normal and pathological. In most cases, senescent cells have been shown to cause degenerative changes primarily through proteins secreted by SASP. Senescent cells can destroy normal tissue structures that are essential for normal tissue function [8]. Senescent cells and SASP can also promote over age-related diseases. For example, indirect evidence indicates that astrocytic aging and associated SASP can promote age-related neurodegenerative disease, leading to cognitive impairment and Alzheimer's and Parkinson's disease. A prominent feature of senescent cells is the senescence-associated secretory phenotype (SASP). Aged cells usually have several pro-inflammatory cytokines (such as interleukin IL6, IL8, tumor necrosis factor (TNF-α) and monocyte chemo attractive protein), growth factor (including platelet Cellular derived growth factor AA (PDGF-AA) [9] vascular endothelial growth factor [VEGF] [10] chemokine, and extracellular matrix-degrading protein metalloproteinase containing matrix (MMP) [11].

Senescent cells have characteristically enlarged and flattened morphology associated with altered gene expression, altered metabolic processes, and protein processing. Furthermore, chromatin structure is rearranged, resulting in transcriptional silencing of growth-promoting genes in heterochromatin clusters called aging related heterochromatin foci (SAHF) [12].

Identification of Senescent Cell and Their Markers:

Senescent cells display several features which can be detected by multiple techniques. Given the heterogeneity of senescent cells and the lack of specificity of the markers, a combination of different techniques is often used and encouraged for the detection of senescent cells.

- **Cell size:** *In vitro*, the enlarged cell body and irregular shape of senescent cells is easily evaluated by regular bright-field microscopy.
- **Cell cycle arrest:** Measurement of the expression level of the CDKIs, p16 and p21.
- **DDR:** The presence of γ-H2AXfoci measured by immunostaining demonstrates continuous and unrepaired DNA damage. It also can be used the measurement of the phosphorylated p53 level.
- **Secretory phenotype:** There is also an altered secretory pattern in senescent cells (senescence associated secretory phenotype). ELISA for cytokines (e.g., IL-1β, IL-6, TNF-α, and IFN-γ are used to detect the protein expression and secretion, particularly IL-6).
- **Apoptosis resistance:** The upregulation of the BCL-proteins Bcl-2, Bcl-w, or Bcl-xL has been used as a marker of senescence. However, it is not yet regularly used.

- **β−GalactosidaseActivity Increases:** One of the first tests used to assess senescence was the senescence-associated β−Galactosidase (SA-β−Gal), the most common marker of lysosomal activity. This marker partly reflects the expansion of the lysosomal compartment due to the production of higher level of β−Galactosidase enzyme. This enzyme can be detected by using substrate X-gal that leads to dark blue staining of senescence cell and staining the cell at pH 6.

Mechanisms Responsible for the Induction of Senescence:

Senescence is triggered by developmental signals or different kinds of stress. Depending on the cell type and intensity and nature of the stress, cells may respond by inducing repair, cell death or senescence.

Cells can undergo senescence in response to various intrinsic (Oxidative damage, telomere attrition, hyperproliferation) or external stimuli (ultraviolet, γ-irradiation, chemotherapeutic drugs), including progressive telomere shortening, changes in telomeric structure, mitogenic signals, oncogenic activation, radiation, oxidative and genotoxic stress, epigenetic changes, chromatin disorganization, perturbed proteostasis, mitochondrial dysfunction, inflammation, and/or tissue damage signals, irradiation, or chemotherapeutic agents, nutrient deprivation.

Currently, senescence is considered a stress response that can be induced by a wide range of intrinsic and extrinsic stimuli, including oncogenic activation, oxidative and genotoxic stress, mitochondrial dysfunction, irradiation, or chemotherapeutic agents. While the defining characteristic of senescence is the establishment of a stable growth arrest that limits the replication of damaged and old cells, many other phenotypic alterations associated with the senescent program are relevant to understanding the pathophysiological functions of senescent cells. For example, senescent cells undergo morphology changes, chromatin remodelling, and metabolic reprogramming, and secrete a complex mixture of mostly proinflammatory factors termed the senescence-associated secretory phenotype (SASP). Here, we review the molecular mechanisms controlling cellular senescence with a special focus on their translational relevance and suitability for identifying and characterizing senescent cells *in vivo*.

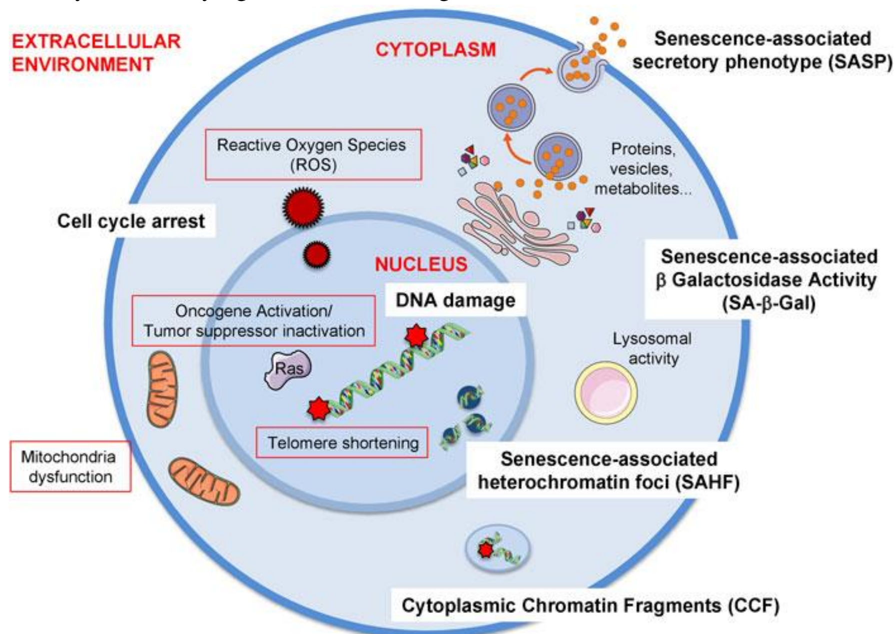

Figure 2: Triggers and biomarkers of cellular senescence

A) Telomere Shortening

Telomeres are heterochromatic repeated sequences of nucleotides at both ends of human chromosomes, consisting of 8-12 kilobases at birth. With each DNA replication, 50-200 base pairs of telomeres are lost from each human cell, due to the inability of DNA polymerase to replicate the whole molecule. Telomeres shorten with each cell division until they reach a critical point. As a result, a DNA damage response (DDR) is elicited, which in turn increases

$p16^{INK4A}$ and $p21^{CIP1}$ cellular levels finally promoting senescence [13]. This type of senescence is called replicative senescence because it originates from the number of replications a cell line undergoes [14-16].

B) Oncogene Activation

Oncogene overexpression and tumour suppressor gene inactivation promote oncogene-induced senescence (OIS). Oncogenes are mutated forms of normal genes present in the human genome, called proto-oncogenes. Under normal circumstances, these genes regulate physiological functions favourable to the cells, but when mutated by gene overexpression or amplification they have the potential to promote cancer development. Tumour suppressor genes code for proteins regulating pathways that contribute to the prevention of cancer development. Thus, their loss of function leads to the loss of these cancer-protective properties, causing cancer. Oncogenes known to be overexpressed in OIS include RAS, BRAF, AKT, E2F1 and cyclin-E. Tumour suppressor genes commonly lost in OIS are PTEN and NF-1 [17-19].

C) Dysfunction

Reactive oxygen species (ROS) is a group of molecules, including hydrogen peroxide (H_2O_2), superoxide ion ($O_2^{\bullet -}$) and hydroxyl radical ($^{\bullet}OH$). They are products of oxidative metabolism in mitochondria, usually scavenged by the enzyme superoxide dismutase (SOD). When mitochondria malfunction, ROS are released causing oxidative damage to mitochondrial and cellular DNA [13, 20]. ROS can also form from the interaction of exogenous factors, such as UV radiation and chemicals from tobacco, and damage cellular DNA. These reactions signal a DDR similar to that caused by telomere shortening, activating $p21^{CIP1}$ and $p16^{INK4A}$ and causing senescence [13].

Cell cycle arrest:

Cell cycle arrest in senescence is largely mediated via activation of either one or both p53/p21 and p16 tumor suppressor pathways. Both these pathways are complex as they involve many upstream regulators and downstream effectors along with varying side branches. Activation of the p53/p21WAF1/CIP1 and p16INK4A/pRB tumor suppressor pathways play a central role in regulating senescence. Several other pathways have recently been implicated in mediating senescence and the senescent phenotype [21-24].

Cellular senescence is different from another form of growth arrest known as quiescence, in that senescence occurs in G1 and possibly G2 phase of the cell cycle as opposed to quiescence which happens in G0. Unlike quiescent cells, senescent cells are nonresponsive to mitogenic or growth factor stimuli; thus, they are unable to re-enter the cell cycle even in advantageous growth conditions. Senescent cells are also distinct from terminally differentiated cells, which are also irreversibly withdrawn from cell cycle. While terminal differentiation is the result of a defined developmental program, which turns undifferentiated precursors into specialized effector cells, senescence is mainly implemented as a cellular stress response.

However, terminally differentiated cells such as neurons, adipocytes, and hepatocytes can also undergo senescence, or at least show senescence-like features, during aging or in response to oncogenic activation or DNA damage. This indicates that the onset of senescence can occur independently of an active cell cycle arrest.

Secretory Phenotype:

Senescent cells characteristically affect their surrounding tissue by secreting substances, grouped under the term senescence-associated secretory phenotype (SASP). This secretion is regulated by pathways involving the protein kinase p38MAPK and the transcription factor nuclear factor kappa beta (NF-κB). The secreted substances include soluble, growth and extracellular matrix (ECM)-remodeling factors, although recently other components such as extracellular vesicles and metabolites have been found [25]. They act by either reinforcing or spreading senescence to surrounding cells and activating immune responses for cell clearance.

Soluble factors include interleukins (IL) 1α, 1β, 6, 7, 8, 13 and 15, and chemokines, such as monocyte chemoattractant proteins (MCP) 2 and 4, macrophage inflammatory proteins (MIP) 1a and 3a, and eotaxin. Such factors recruit immune cells to clear senescent cells. Growth factors include, among others, insulin-like growth

factor (IGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF) and angionenin. These factors signal pathways regulating cell growth and angiogenesis, and in such microenvironments, they contribute to senescence [10]. ECM-remodeling factors, such as matrix metalloproteinases (MMP), ADAMTS proteins and integrins also contribute to senescence. They act by altering ECM components, like collagen, and promote ECM degradation. This disrupts the normal crosstalk between cells and disorganizes their physical attachments, contributing to senescence. ECM disruption also aids in immune cell recruitment [26-27].

Extracellular vesicles are lipid membrane vesicles released by either pinching of the cytoplasmic membrane of the cell or formed upon activation of the endocytic pathway [28]. They contain a variety of proteins, nucleic acids, metabolites and lipids that decide their course of action and functionality in recipient cells [29-30]. They have been recently identified as part of the SASP mediating both paracrine senescence in different contexts [22] and rejuvenation of different tissues in aged mice [25, 30-31].

Senescence as a Therapeutic Target

Senescent cells actively contribute to aging and age-related pathologies. Senescent cells thus represent attractive therapeutic targets to treat certain conditions, restore health span, and possibly extend life span. Indeed, the proliferative arrest, increased expression of lysosomal b-galactosidase, and resistance to apoptosis are some key features of senescent cells that are currently being exploited in the development of serotherapy. One class of senolytic drugs are rationally designed small molecules that disrupt the anti-apoptosis pathways activated in senescent cells, forcing their elimination through apoptosis [32]. This strategy offers an alternative to simply targeting, for example, p16INK4A-expressing cells, as p16INK4A might also play important roles in other physiological processes.

Some of the first senolytic drugs described, quercetin and dasatinib, induce apoptosis of senescent cells when administered in combination and thereby alleviate morbidity in normal, aging and progeroid mice [33]. Similarly, the small molecule ABT263, an inhibitor of anti-apoptotic molecules BCL-2 and BCL-xL, rejuvenates the hematopoietic and muscle stem cell (HSC and MuSC) compartments and reverses lung damage of sublethally irradiated mice by inducing apoptosis of irradiation-induced senescent cells [34]. An alternative strategy for the elimination of senescent cells was recently described in a trichothiodystrophy (TTD) mouse model of accelerated aging. In these mice, disruption of the p53-Foxo4 interaction with a cell-penetrating peptide FOXO4-DRI induces apoptosis of senescent cells, possibly due to the mitochondrial translocation of p53 from the nucleus. Consequently, this peptide opposes the aging-associated decline of renal and liver function [35]. Another approach for senotherapy takes advantage of the fact that senescent cells express high levels of lysosomal beta galactosidase. Nanoparticles coated with galacto-oligosaccharides are thus preferentially taken up by senescent cells, and when such nanoparticles encapsulate cytotoxic drugs, they effectively eliminate senescent cells in vitro and in vivo. This strategy to eliminate senescent cells has been demonstrated to promote tumor regression and resolve pulmonary fibrosis in animal models [8]. Together, these studies provide the proof-of-principle evidence that pharmacological elimination of senescent cells could have rejuvenating effects in humans. Modulation of the SASP represents another avenue for the development of senotherapeutics. Indeed, specific disruption of SASP components represents an alternative chemotherapeutic strategy for cancer and other age-related disorders, as this would prevent the reprogramming of adjacent cells within the affected tissue and thus reduce disease progression. In this context, another approach to modulate the senescent phenotype uses small molecules to target critical regulators of the SASP such NF-kB, the JAK/STAT pathway, and mTOR (i.e., senomorphics). It is hypothesized that the modulation of these pathways could bring about beneficial effects such as reductions in chronic inflammation, inhibition of paracrine senescence, and restoration of tissue function (Soto-Gamez and Demaria 2017). However, due to the dynamic and compositional heterogeneity of the SASP, as well as the pleiotropic effects of its components, a road for the development of SASP-specific therapeutics is not evident. More studies on the dynamics and regulation of the SASP in response to diverse senescence inducers are required to fulfill the potential of SASP-specific therapies in the treatment of age-related pathologies.

References

[1]. Noren N., Evans MK., Techniques to Induce and quantify cellular senescence. 2017; 123:55533.

[2]. Vicencio JM., et al. Life, death and burial: multifaceted impact of autophagy. BiochemSoc Trans. 2008; 36:786-790.

[3]. Coppe J.P., Patil C. K., Rodier F., Sun Y., Muñoz D. P., Goldstein J., Nelson, P. S., Desprez, P.Y., &Campisi, J. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic Ras and the p53 tumor suppressor. PLoS Biology. 2008; 6:301.

[4]. Fitsiou E., et al., Cellular senescence and the senescence-associated secretory phenotype as drivers of skin photoaging. 2021; 141:1119-1126.

[5]. Hayflick P., Moorehead P.S., The serial cultivation of human diploid cell strains. Exp. Cell Res 1961; 25:585-621.

[6]. D'Addadi Fagagna F., et al. A DNA damage checkpoint response in telomere-initiated senescence 2003; 426:194-198.

[7]. Herbig U., et al. Telomere shortening triggers senescence of human cells through a pathway involving ATM 2004; 14:501-513.

[8]. Muñoz-Espín D., Serrano M., Cellular senescence: from physiology to pathology. 2014; 15:482-496.

[9]. Demaria M., et al. An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. Dev Cell. 2014; 31(6):722-733.

[10]. Coppé J.P., Desprez P.Y., Krtolica A. and Campisi J. The Senescence-Associated Secretory Phenotype: The Dark Side of Tumor Suppression. Annu. Rev. Pathol. Mech. Dis. 2010; 5:99-118.

[11]. Gorgoulis et al., Cellular Senescence: Defining a Path Forward 2019; 179:813-827.

[12]. Narita M., Nunez S., Heard E., Lin A.W., Hearn S.A., Spector D.L., Hannon G.J., Lowe S.W., Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. Cell. 2003; 113:703-716.

[13]. Di Micco R., Krizhanovsky V., Baker D., and d'Adda di Fagagna F. Cellular Senescence in Ageing: From Mechanisms to Therapeutic Opportunities. Nat. Rev. Mol. CelBiol 2021; 22:75-95.

[14]. Deng Y., Chan S. S., and Chang S. Telomere Dysfunction and Tumour Suppression: The Senescence Connection. Nat. Rev. Cancer 2008; 8:450-458.

[15]. Fafian-Labora J. A., and O'Loghlen A. Classical and Nonclassical Intercellular Communication in Senescence and Ageing. Trends Cel Biol. 2020; 30:628-639.

[16]. Fafian-Labora J. A., Rodriguez-Navarro J. A. and O'loghlen, A. Small Extracellular Vesicles Have GST Activity and Ameliorate Senescence-Related Tissue Damage. Cel Metab. 2020; 32:71-86.

[17]. Gil J., and Peters G. Regulation of the INK4b-ARF-INK4a Tumour Suppressor Locus: All for One or One for All. Nat. Rev. Mol. CelBiol 2006; 7:667-677.

[18]. Perez-Mancera P. A., Young A. R. J., and Narita M. Inside and Out: The Activities of Senescence in Cancer. Nat. Rev. Cancer 2014; 14:547-558.

[19]. Lee S., and Schmitt C. A. The Dynamic Nature of Senescence in Cancer. Nat. CelBiol 2019; 21:94-101.

[20]. Desdín-Micó G., Soto-Heredero G., Aranda J. F., Oller J., Carrasco E., Gabandé-Rodríguez E., et al. T Cells with Dysfunctional Mitochondria Induce Multimorbidity and Premature Senescence. Science 2020; 368:1371-1376.

[21]. Bhaumik D., Scott G. K., Schokrpur S., Patil C. K., Orjalo A. V., Rodier F., et al. MicroRNAs miR-146a/b negatively modulate the senescence-associated inflammatory mediators IL-6 and IL-8. 2009; 1:402-411.

[22]. Borghesan M., Fafián-Labora J., Eleftheriadou O., Carpintero-Fernández P., Paez-Ribes M., Vizcay-Barrena G., et al. Small Extracellular Vesicles Are Key Regulators of Non-Cell Autonomous Intercellular Communication in Senescence via the Interferon Protein IFITM3. Cel Rep. 2019; 27:3956-3971.

[23]. Chen Q., Ames BN. Senescence-like growth arrest induced by hydrogen peroxide in human diploid fibroblast F65 cells. 1994; 91:4130-4134.

[24]. Chen Q., Fischer A., Reagan JD., Yan LJ., Ames BN. Oxidative DNA damage and senescence of human diploid fibroblast cells. 1995; 92:4337-4341.

[25]. Fafián-Labora, J. A., & O'Loghlen, A. (2020). Classical and nonclassical intercellular communication in senescence and ageing. *Trends in Cell Biology*, *30*(8), 628-639.

[26]. Rapisarda V., Borghesan M., Miguela V., Encheva V., Snijders A.P., Lujambio A., et al. Integrin Beta 3 Regulates Cellular Senescence by Activating the TGF-β Pathway. Cel Rep. 2017; 18:2480-2493.

[27]. Fane M. and Weeraratna, A.T. How the Ageing Microenvironment Influences Tumour Progression. Nat. Rev. Cancer 2020; 20:89-106.

[28]. Van Niel G., D'angelo G. and Raposo, G. Shedding Light on the Cell Biology of Extracellular Vesicles. Nat. Rev. Mol. Cel Biol 2018; 19:213-228.

[29]. Tkach M. and Théry C. Communication by Extracellular Vesicles: Where We Are and where We Need to Go. 2016; Cell 164:1226-1232.

[30]. O'Loghlen A. The Potential of Aging Rejuvenation. Cell Cycle 2022; 21 (2):111-116.

[31]. Yoshida M., Satoh A., Lin J.B., Mills K.F., Sasaki Y., Rensing N., et al. Extracellular Vesicle-Contained eNAMPT Delays Aging and Extends Lifespan in Mice. Cel Metab. 2019; 30:329-342.

[32]. Van Deursen, J. M. (2014). The role of senescent cells in ageing. *Nature*, *509*(7501), 439-446.

[33]. Zhu J., Woods D., McMahon M., Bishop JM. Senescence of human fibroblasts induced by oncogenic Raf. 1998; 12:2997-3007.

[34]. Chang, J., Wang, Y., Shao, L., Laberge, R. M., Demaria, M., Campisi, J., ... & Zhou, D. (2016). Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. *Nature medicine*, *22*(1), 78-83.

[35]. Baar, M. P., Brandt, R. M., Putavet, D. A., Klein, J. D., Derks, K. W., Bourgeois, B. R., ... & de Keizer, P. L. (2017). Targeted apoptosis of senescent cells restores tissue homeostasis in response to chemotoxicity and aging. *Cell*, *169*(1), 132-147.

[36]. Soto-Gamez, A., & Demaria, M. (2017). Therapeutic interventions for aging: the case of cellular senescence. *Drug Discovery Today*, *22*(5), 786-795.