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REVIEW ARTICLE

Senescence-Associated Secretory Phenotype (SASP) involvement in the development of cancer, aging and age related diseases

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Abstract

Cellular senescence is defined as the physiological program of terminal growth arrest; in mammals it is an important tumor-suppressor mechanism since it stops premalignant cell proliferation. However, senescence also contributes to the decline associated to aging and the development of several diseases. This is explained by the fact that senescent cells secrete diverse molecules, which compromise the cellular microenvironment, and altogether are referred as senescent-associated secretory phenotype (SASP). The SASP is composed by cytokines, chemokines, growth factors, proteases, etc., whose function is to maintain the antiproliferative state and promote senescent cell clearance by the immune system. Nevertheless, over time, and particularly during old age, SASP might stimulate proliferation and premalignant cell transformation. The multifunctional roles of SASP would depend on the cell type and their physiological nature. Therefore, relying on the biological context, SASP could be beneficial and participate in the repair and regeneration processes, or detrimental and induce degenerative pathologies and cancer. (Gac Med Mex. 2015;151:460-8)

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Historical antecedents

Approximately a century ago, one of cell biology paradigms was that cells cultured *in vitro* could live and replicate endlessly, provided they were supplemented with adequate media and nutrients. This fact had a marked implication in the study of aging, since cultured cells could apparently live more years than the organism they came from.

However, in the decade of the 60's, Hayflick and Moorhead demonstrated that cells in primary culture had only a limited number of replications and could not proliferate indefinitely, a fact that generated a new paradigm and broke up with the previous one. Now, when a primary culture ceases its cell proliferation, it is said to have reached the Hayflick limit[1,2], and this phenomenon is known as cell senescence. However, since it is a process related to the number of cell replications (age of the culture), for many years it was only associated to the aging phenomenon.

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In 1995, Campisi's group demonstrated the presence of senescent cells *in vivo,* which revealed that it was not a phenomenon occurring exclusively *in vitro,* and senescence was proven to occur not only during the process of aging, but to occur naturally and throughout the entire life of organisms[3,4].

Cell senescence characteristics

Cell senescence is defined as the stage where cells have reached the peak of their proliferative capability and are not able to further replicate[2]. It is characterized by the irreversible arrest of the cell cycle in the G0/G1 phase[5]. Senescent cells show morphological alterations, such as an increased size with a flattened appearance and a large amount of vacuoles. At the physiological level, they have been shown to be metabolically active, but they show a disturbed gene expression, which entails an alteration in protein expression, associated with the previously described morphological changes, together with a lack of response to mitogenic and/or apoptotic stimuli[6]. In addition, this type of cells shows particular modifications at the heterochromatin level known as senescent-associated heterochromatin foci[7]. Another peculiar characteristic of senescent cells is that they enhance the expression of the beta-galactosidase enzyme and, although the reason for this phenomenon is not clearly understood, the activity of this enzyme is used as a senescent status marker[3].

Telomere shortage has been traditionally assumed to be the signal for cells to stop proliferating. It is a well known fact that with each cell replication there is a gradual loss of DNA at the chromosomal extremes, a phenomenon known as telomere erosion[8]. This occurs during the S phase of the cell cycle, since DNA polymerases are unidirectional and unable to synthesize the DNA portion is acting as a primer. Telomere erosion generates a persistent DNA damage response (DDR)[5,9,10], which is an important signal to prevent progression of the cell cycle, since it induces the expression of cell cycle-inhibitor molecules, such as p16 and p21[10]. Senescence induced by this mechanism is known as replicative senescence (RS).

In mammals, DDR can be initiated by the ataxia telangiectasia mutated (ATM) and ataxia telangiectasia Rad3-related (ATR) protein kinases. ATM is recruited and activated by proteins that bind to DNA sites where the double chain has been broken, whereas ATR is recruited and activated by the A replication protein. Once activated, ATM and ATR activate their targets, CHK2 and CHK1, respectively, whose function is to activate transcription factor p53[11].

In response to DDR, p53 can regulate processes such as DNA repair, cell cycle arrest, senescence and apoptosis[12]. One of p53 target genes is the cell-cycle inhibitor p21[13], which inhibits CDK2/cyclin E and CDK4/6/cyclin D complexes, important for cell cycle progression[14,15], thus inducing the typical proliferation arrest of senescent cells.

Now it is accepted that there are other pathways through which cells can enter into senescence and stop proliferating, regardless of the number of replications and, therefore, of telomere shortage. Stimuli and stressors able to induce cell senescence are very diverse: exposure to oxidative stress[16], exposure to ultraviolet and gamma radiation[17], hyperoxia[18], oncogene overexpression (for example, Ras)[19,20], deterioration of certain protein degradation or alteration of cellular cleaning mechanisms like autophagy[21] or proteosome inhibition[22]. Senescence induced by any of these stimuli is known as stress-induced premature senescence and has a phenotype that is similar to that observed in RS[18,23,24].

Cell senescence: pleiotropic antagonism, a double-edged blade

As previously mentioned, for many years, the term *senescence* defined only a series of changes associated with aging; nevertheless, now it refers to a program of signal transductions that lead to irreversible arrest of the cell cycle and that are accompanied by a characteristical phenotype[25]. Currently, *in vivo* senescence is thought most likely not to be induced by telomere shortage, as there are other factors (such as those previously mentioned) that can induce senescence long before the cell reaches the Hayflick limit[26].

In mammals, senescence plays a highly important role in the preservation of tissue functionality, especially as a tumor suppressor, since it limits the proliferation of damaged cells potentially at risk for neoplastic transformation[5,27,28], although it can contribute to the aged phenotype as well. This apparent contradiction can be explained by pleiotropic antagonism, one of the main evolutive theories on aging, which establishes that certain processes that have been selected due to their beneficial effects at early ages have deleterious effects at older ages. This is rarely observed in wild populations, due to the low number of individuals reaching old age, but it is commonly observed in humans[29]. In the case of cell senescence, at early ages, it has a

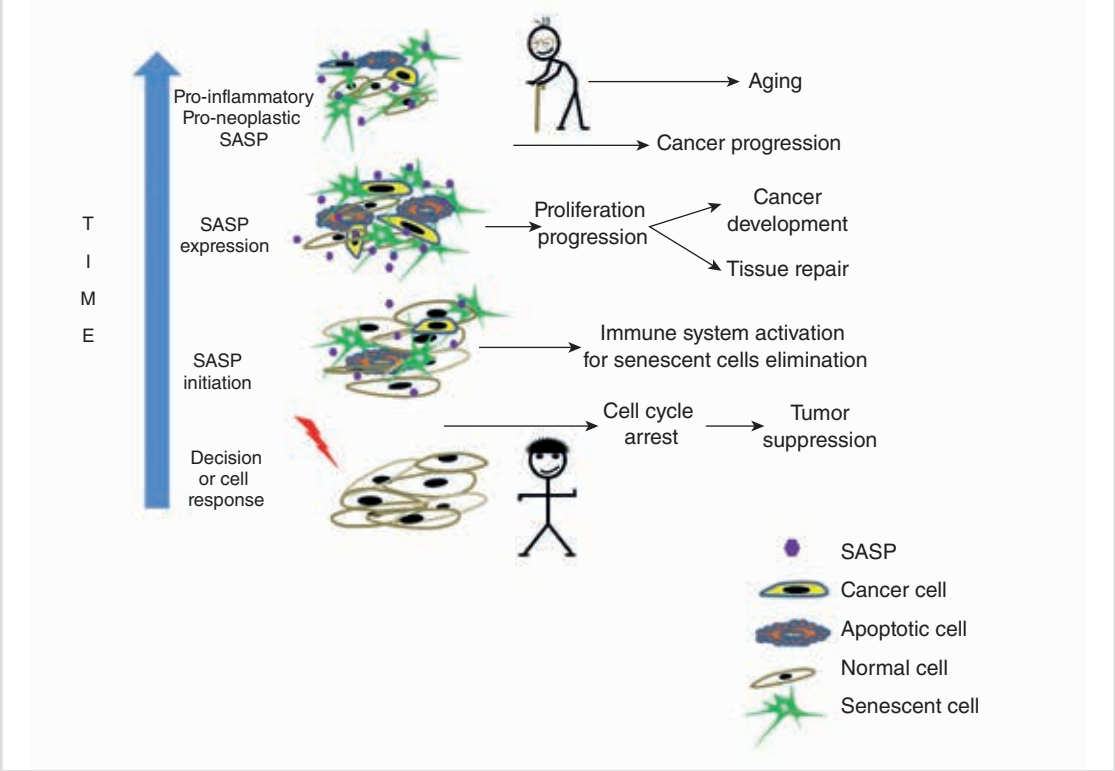

Figure 1. *Transformation of SASP over time. In a first stage, damaged cells or cells subjected to certain types of stress exhibit a cell cycle arrest that is reflected as a tumor suppressor event, since it prevents damaged cells proliferation. However, senescent cells secrete different molecules, such as cytokines, chemokines, metalloproteinases, etc., which together are known as SASP. In addition to being a senescent phenotype perpetuation autocrine event, SASP also attracts immune cells that destroy senescent cells. However, if these cells are not removed, some SASP components can promote cell proliferation, either for tissue repair or cancer development. A late manifestation of SASP is the expression of proteins associated with chronic inflammatory response and, therefore, senescent cells accumulation with chronic SASP levels might influence on organisms' health by generating an aging phenotype and promoting different diseases.*

beneficial effect by suppressing malignant cells that potentially generate tumors; however, at older ages, senescent cells accumulation contributes to aging-associated tissue deterioration[30].

The functional alteration occurring in tissues due to senescent cells accumulation has been explained by pro-inflammatory components secreted by these cells and that compromise the cell microenvironment. The group of secreted molecules is referred to as senescence-associated secretory phenotype (SASP).

SASP essential functions have been suggested to be related to perpetuating the antiproliferative status, in addition to generating signals that promote senescent cell elimination by the immune system[30]. However, over time, and particularly in old organisms with a reduced immune response, SASP can favor cancer and other diseases by secreting factors that rarefy the cell microenvironment by stimulating pre-malignant cells proliferation and transformation; therefore senescence is, paradoxically, a double-edged blade (pleiotropic antagonism), since it suppresses the onset of cancer at early stages of life, but eventually it promotes the onset of neurodegenerative pathologies such as Alzheimer[31] (Fig . 1).

SASP

The SASP is characterized by an increase in the secretion of approximately 40-80 factors involved in different intra-cellular signaling pathways[32,33]. The SASP includes some soluble and insoluble families of factors that modulate surrounding cells, since they activate membrane surface receptors and their corresponding signal transduction pathway and can lead to multiple diseases (Fig. 2). Factors can be divided in two main categories: soluble or protein signaling factors (interleukins, chemokines, growth factors and proteases)

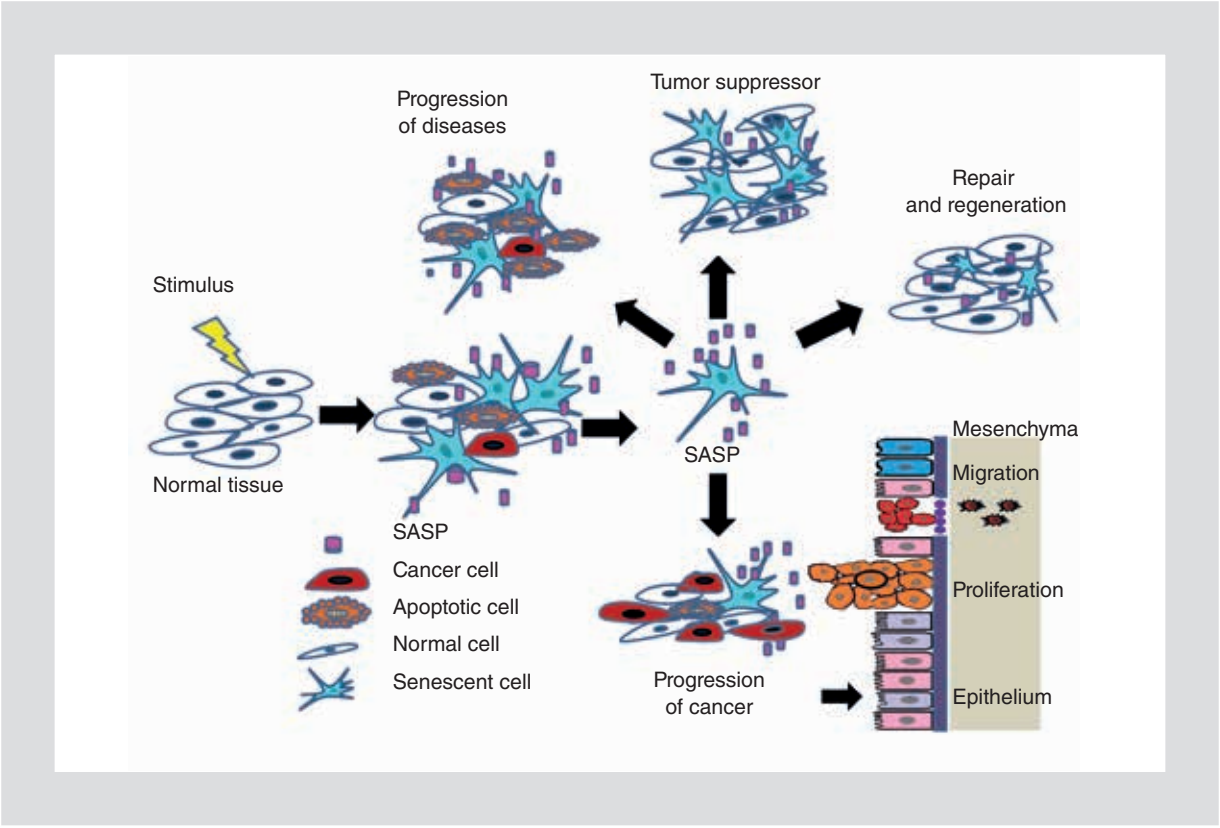

Figure 2. *Senescent cells biological activity. The molecules comprising the SASP can be different depending on cell type, tissue and form of senescence induction, and change in a time-dependent manner; therefore, they can have different effects on adjacent cells, such as cell-proliferation inhibition (tumor-suppressor mechanism), damaged tissue repair and regeneration, tumor progression and EMT; in addition, the establishment of a chronic pro-inflammatory environment will promote the onset of different diseases.*

and insoluble or non-protein components (reactive oxygen species [ROS])[34].

Soluble factors

It is important mentioning that the composition of cytokines, chemokines and growth factors in the SASP can vary depending on the tissue and cell type. Moreover, recent results from our laboratory[35] have shown differences depending on the route of induction by means of which cells reach the senescence stage. In this section, we will discuss some of the most important and general protein components that constitute the SASP.

In the first place, in almost all studies on SASP, the secretion of interleukin 6 (IL-6), which also has a pleiotropic function, has been found to be increased. The secretion of this interleukin has been shown to be significantly increased in human and mouse keratinocytes, melanocytes, monocytes, fibroblasts and epithelial cells after DNA damage when senescence is induced with oncogenes[36-38]. Another interleukin present in SASP is interleukin 1 (IL-1); both IL-1α and IL-1β are over-expressed and secreted by fibroblasts[39,40], senescent epithelial cells[41] and chemotherapy-induced senescent epithelial cells[42]. Interleukin 8 (IL-8) expression depends on IL-1α secretion, indicating there is regulation of the SASP components. IL-8 is over-expressed in many types of senescent cells together with the Growth-Related Oncogene (GRO) α and β.

Members of the chemokines family that usually are overexpressed in senescent cells are: membrane co-factor protein (MCP) 2, 4 and 1, HCC-4, eotaxin-3, MIP-3α and 1α (macrophage inflammatory protein); MCP-3 is overexpressed by senescent stellate liver cells and by prostate and skin fibroblasts.

Insulin-like growth factor (IGF) bound to its receptor can contribute to the effects exerted by senescent cells on their microenvironment, since senescent endothelial and epithelial cells and fibroblasts have been found to express high concentrations of IGF-bound proteins (IGFBP), including IGFBP-2, 3, 4, 5 and 6[43,44]. There are other additional soluble factors associated with SASP that are secreted at high concentrations by senescent fibroblasts, including some colony-stimulating

factors (CSF), including macrophage CSF (GM-CSF) and granulocyte CSF (G-CSF), as well as epithelial growth factor, fibroblast growth factor[45] and intercellular adhesion molecule 1.

In addition to signaling cytokines and growth factors, senescent cells also secrete high levels of some extracellular matrix metalloproteinases (MMP). In particular, human and mouse senescent fibroblasts secrete stromelysin 1 and 2 (MMP-3 and 10, respectively) and collagenase 1 (MMP-1)[46,47]. In some cases, MMP-1 and 3 can also regulate the activity of soluble factors present in the SASP[48]. For example, these MMPs can decrease MCP-1, 2 and 4 and IL-8[49].

Another family of proteases involved in carcinogenesis and present in the SASP is the family of serine proteases and regulators of the plasminogen activation pathway, tissue activators or urokinases (uPA or tPA), the uPA receptor (uPAR) and serine protease inhibitors (plasminogen activation inhibitor [PAI] 1 and 2)[50].

SASP proteases can have three main effects: they dissociate proteins (e.g., they dissociate membrane-associated receptors by making them soluble), degrade signaling molecules and transform the extracellular matrix. These effects are considered to be potent mechanisms by means of which senescent cells can modify the tissue microenvironment.

Nonprotein components

Main nonprotein components of SASP are ROS, in addition to some ions and micro-RNAs. Senescent cells have been shown to release nitric oxide and ROS, since alterations have been detected in endothelial nitric oxide synthase and superoxide dismutase activities[51-55]. These molecules can increase aggressiveness in cancer cells and promote aging and age-related diseases as well[56,57].

SASP inflammatory components regulation

In general, senescent cells gene expression profiles (mRNA), as determined by transcription analysis, are similar to those of the secreted proteins, as determined by antibody microarrays. This finding suggests that SASP is regulated at the transcriptional level. However, since changes in gene expression that are produced during senescence are broad, transcriptional control can also occur at the epigenetic level, i.e, through modifications in the chromatin organization. In fact, the most significant chromatin modifications occur during senescence[7,28,58-60].

The expression of many of the SASP components depends on transcription factors NF-κB and C/EBPβ, which increase their activity in senescence[61]. Mutant mice lacking C/EBPβ have been reported to decrease the expression of IL-6 and IL-8, which are present in a predominant manner in SASP[38], whereas when mutants for NF-κB are used, 75% of SASP factors are decreased[27,62].

The signaling cascade that is activated in response to DNA damage (DDR), and that has been previously disscused, has been proposed to be essential for the increased secretion of a subset of SASP factors, including IL-6 and IL-8[10,63]. This persistent DDR response is necessary for SASP to occur. In contrast, the decrease in the DDR cascade components, especifically ATM, NBS1 or CHK2, prevents the increase of secreted proteins, in particular IL-6, IL-8 and the GRO family. However, the DDR response is not considered to be the only SASP regulator, since it is activated immediately after injury is sustained, whereas SASP, just like other aspects of the senescent phenotype, such as the beta-galactosidase activity, takes several days to become apparent[10]. Therefore, DDR is important, but not sufficient, and this suggests that there have to be other elements cooperating with DDR to induce SASP. One of such events is p38MAPK, which is increased after DNA damage.

In SASP, there are also positive feedback components. IL-1α regulates its own synthesis, mediated by its receptor and, therefore, it represents a form of autocrine positive-feedback[64,65]. IL-1α decrease by interferent RNA in senescent cells has been observed to markedly reduce IL-6 and IL-8 expression and secretion. Furthermore, similar results are reported when an IL-1 antagonist or a neutralizing antibody to the receptor (IL-1R) are used, which demonstrates that sustained stimulation of IL-1R bound to IL-1α is required to maintain senescence associated with extracellular IL-6 and IL-8 increase[66,67].

SASP and cancer

Up to this moment, it is clear that the factors comprising the SASP confer senescent cells a powerful ability to modify extracellular microenvironment, since they can trigger signaling pathways that will modify the responses in adjacent cells[68,69] (Fig. 2). Analyzing and specifying SASP biological activity is quite complex because some factors favor the deterioration and damage effect, while others may even have beneficial effects.

As mentioned in the previous section, SASP is mainly a DDR-associated trait of senescent cells, since cells entering into senescence only due to $p21^{Cip}$ or $p16^{INK4a}$ proteins exogenous overexpression have been observed not to express a SASP, because in spite of having senescent cells characteristics and requiring for p53 to stop their proliferation, it appears that these conditions are not sufficient for SASP to be secreted[27]. In contrast, cells lacking functional p53 have been reported to markedly secrete high levels of many SASP components[70]. Conversely, cells entering into senescence due to DNA damage do develop a SASP and show dysfunctional telomeres, epigenetic alterations, mitogenic signals, oxidative stress and other senescence-inducing stimuli. This suggests that SASP could be fulfilling the function of warranting for damaged cells to communicate their compromised status to neighboring cells preparing the tissue for repair or regeneration. Other important function of SASP is the stimulation of the immune system to eliminate senescent cells, thus preventing their accumulation, as well as wound healing repair.

However, a large amount of SASP components directly or indirectly promote chronic inflammation[71], and this is a cause associated with practically all age-related diseases, both degenerative and hyperplastic[5]. In particular, chronic inflammation has been reported to play an important role in cancer early stages[34]. SASP can stimulate cell proliferation and differentiation[72], as well as angiogenesis, since it stimulates the formation of new vessels and the epithelial-mesenchymal transition (EMT); therefore, it will favor metastasis in premalignant cells[30,73].

For example, senescent fibroblasts secrete amphiregulin and GROα, which induce premalignant epithelial cells proliferation, together with IL-6 and IL-8, which stimulate them to invade the basement membrane[68]. Additionally, mesothelial cells and fibroblasts have been reported to secrete vascular endothelial growth factor (VEGF), which stimulates angiogenesis, favoring endothelial cells invasion and migration[74]. When fibroblasts reside in a tumor microenvironment, they turn into contractile myofibroblasts referred to as CAF. Both CAFs and senescent cells show alterations in the autophagy process, which leads to tumor progression[75]. In other studies, breast premalignant epithelial cells have been reported to be induced to proliferate by stromal senescent cells, whereas in the oral cavity, these same stromal senescent cells have been observed to stimulate epithelial cells growth by decreasing their integrity and disrupting differentiation. In the prostate, senescent fibroblasts stimulate epithelial cells hyperproliferation and induce EMT in surrounding epithelia[76].

However, and apparently in a conflicting manner, SASP can also prevent the development of cancer, since senescent keratinocytes have been reported to secrete the anti-angiogenic factor maspin[77]. Furthermore, human senescent melanocytes are known to secrete IGFBP-7, which induces senescence in non-senescent melanocytes and apoptosis in some melanoma cell lines[78], thus preventing damaged cells proliferation. With regard to IL-6 and IL-8, which had already been discussed as malignancy inductors, there are reports that, together with the PAI-1 inhibitor, they can promote tumor suppression by inducing senescence through oncogenes or oxidative stress[37]. All this demonstrates the multifunctional and pleiotropic role of SASP, which largely depends on the cell type receiving it and the status it presents.

SASP in aging and associated diseases

Aging can be understood as the progressive deterioration of biochemical, functional and structural capacities of organisms. Recently, chronic inflammation has been incorporated as a predominant and recurrent factor related to the process of aging. Such was the case, that the term “inflamm-aging” has been coined to describe the role played by inflammation in deterioration and old age-associated diseases, which is correlated with the fragility syndrome[79]. Interestingly, among the main inflammation markers associated with neurodegenerative diseases such as Alzheimer[80], depression[81], atherosclerosis[82], cancer[27], diabetes[83], arthritis, osteoporosis and renal failure[78], etc., there are several components of SASP, with IL-6 and tumor necrosis factor alpha (TNF-α) elevated levels standing out, as well as other chemokines and cytokines here described.

There is a large body of evidence strongly supporting that cell senescence has a fundamental role in aging and related conditions, since senescent cells tissue accumulation throughout life and SASP components secretion have been observed to damage tissues and produce dysfunction both locally and in the entire body.

As previously mentioned, the functions of SASP include activation of the immune system in order to promote senescent cells elimination and this way prevent their accumulation in tissues. However, during the process of aging, senescent cells increase and permanence in the body seem to be driven, among other causes, by a decrease in tissue macrophage response during the process of aging[71]. Apparently, the presence of macrophage

-recruiting chemokines, such as monocyte chemoattractant protein-1 (MCP-1), which is part of the SASP, is not sufficient to eliminate the senescent cells. On the other hand, chronic exposure to IL-6 has also been reported to inhibit the macrophage function[84]. In recent studies, some SASP components, such as TNF-α, IL-6, MMP, MCP-1 and IGFBP, have been found to increase in multiple tissues during the process of aging[71]; for example, in the adipose tissue, the expression of IL-6, IGFBP-2 and PAI-1 is increased in senescent cells[85].

Local effects of SASP on tissues or specific components of SASP are implied in a wide range of age-related diseases *in vivo.* For example, senescent endothelial cell SASP represents an important risk factor for age-related vascular calcification through the development of atherosclerotic lesions[86]. Senescent osteoblasts, in addition to generating more oxidative stress, have been found to secrete SASP components that alter the microenvironment and lead to the development of osteoporosis and arthritis in old age[87].

Another example is the effect of SASP expression by astrocytes, both *in vitro* and in cells isolated from aged brain, which initiate or contribute to neuroinflammation[88], a basic characteristic of many neurodegenerative diseases that leads to cognitive and motor deterioration during the aging process[89]. In the case of diabetes, the loss of b-cells regenerative capability is present in the rodent and human aging process due to a decrease in the number of functional cells and presence of senescent cells. In the pancreatic islets, protein $p16^{INK4a}$ plays an important and determinant role, since mutants lacking $p16^{INK4a}$ have been found to increase b-cells proliferation[90].

In spite of the deleterious effects of SASP contributing to diseases during the process of aging, new findings suggest that aging is a modifiable risk factor and that delaying age-related diseases is viable by modulating basic mechanisms of this process. Up to this moment, genetically-modified mice models have been generated, which allow for the causal relationship between cell senescence, SASP and age-related conditions, such as cardiovascular diseases and diabetes, to be dissected. For example, in studies conducted with genetically modified mice, a strategy was designed to specifically eliminate $p16^{INK4a}$-positive senescent cells, with senescent cells elimination from adipose, musculoskeletal and eye tissue being found to delay the age-related diseases phenotype[85]. Similarly, induction of the telomerase catalytic subunit, hTERT, was found to reverse aging functional deterioration in the third generation of a mutant mouse[91-93] and the use of hTERT as gene therapy in an old mouse delayed aging and prolonged the life span[94].

Conclusions

Cell senescence is a highly important event during mamals lifespan, since at an early stage it allows for them to maintain the good functioning of tissues by stopping the proliferation of potentially malignant cells (tumor suppressing effect), but at a late stage, senescent cells accumulation contributes to deterioration during the process of aging and even can promote a large number of diseases, with cancer standing out. This dual role of senescence can be explained by pleiotropic antagonism, which establishes that some processes characterized for their beneficial effects at early ages, produce deleterious effects in later life[29,95].

So, the multifunctional and pleiotropic role of SASP is clearly established and it will largely depend on the type of cell receiving it and its status; hence, depending on the biological context, SASP can be beneficial, by participating in repair and regeneration processes, or deleterious, by promoting degenerative and hyperplastic diseases.

Currently, a large part of the world population has been able to prolong its lifespan, but many of these individuals are also facing years of disability marked by multiple chronic and degenerative diseases, fragility and loss of independence. These conditions seriously disturb social stability and increase health costs[96]. Most of these alterations have been associated with inflammation and disturbance of the cellular microenvironment promoted by SASP; therefore, cell senescence modulation could become a potential pro-longevity strategy. Strategies to achieve this might include selected senescent cells elimination and senescent cells epigenetic reprogramming in order to modify the SASP secretion activity.

Clearly, clinical practice would be transformed if treatments based on the knowledge of the cell senescence mechanisms and the SASP could transform the relationship between key aging processes and chronic conditions, turning aging into a modifiable factor[73], which would result beneficial for the population, since it would be possible to reach older ages, but decreasing age-related diseases and complications.

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