

00013

Cellular senescence as a pathogenic factor and potential therapeutic target in osteoporosis

Lorena Pena Larrea¹, Manuela de Blas Rodríguez², Manuel Naves Díaz³, Carlos Gómez Alonso³

¹Department of Traumatology and Orthopedic Surgery. Hospital Universitario San Agustín de Avilés. Avilés, Asturias. Spain. ²Clinical Management Unit of Bone Metabolism. Hospital Universitario Central de Asturias. Oviedo, Spain. ³Clinical Management Unit of Bone Metabolism. Hospital Universitario Central de Asturias. Grupo de Metabolismo Óseo, Vascular y Enfermedades Inflamatorias Crónicas. Instituto de Investigación Sanitaria del Principado de Asturias (ISPA). RICORS 2040 - Enfermedad Renal del ISCIII. Oviedo, Spain

Authors: Lorena Pena Larrea and Manuela de Blas Rodríguez are the lead authors of this article.

Conflicts of interest: the authors declare no conflict of interest.

Received:

Accepted: 04/18/2023

Correspondence: Lorena Pena Larrea. Department of Traumatology. Hospital San Agustín de Avilés. Cam. de Heros, 6. 33401 Avilés, Asturias. Spain

e-mail: lorenapena89@gmail.com

ABSTRACT

Cellular senescence is a process induced by various types of stress that irreversibly cause cell cycle arrest and changes to the characteristics and functionality of cells, as well as the acquisition of a secretory phenotype that generates a pro-inflammatory environment. While, in certain contexts, it is beneficial for tissues and promotes organism development, senescence is a cellular fate implicated in the process of aging and age-related degenerative conditions. Senolytics are drugs that specifically eliminate senescent cells, and senomorphics are drugs that suppress their senescence-associated secretory phenotype (SASP) without inducing cell death. Therefore, therapeutic strategies targeting senescent cells (senolytics and senomorphics) as an underlying mechanism of aging emerge as an alternative with great potential to fight age-related diseases as a whole rather than individually. One of these conditions is osteoporosis where it has been experimentally described that drugs such as zoledronic acid have effects on preosteoblasts and act on senescent cells extending survival and opening up the possibility of treating age-related diseases with drugs already used in practice, which may have effects beyond the bone itself and increase overall survival. In this study, a review will be conducted in this rapidly growing field in recent years of undeniable translational interest.

Keywords: Senescence. Osteoporosis. Senolytics. Senomorphics. Bone. Frailty. Senescence-associated secretory phenotype (SASP).

INTRODUCTION

The remarkable increase in life expectancy since the mid-20th century has led to a rapid aging of the population (1). Globally, the population over 65 years old is growing at a faster pace compared to other population segments. As a matter of fact, it is estimated that by 2050, the overall number of octogenarians will triple the numbers from 2019 (2). This increase in longevity, an achievement of improved life conditions and medicine itself, is accompanied by an increase in the burden of chronic diseases (cardiovascular, musculoskeletal, neoplastic, and neurological) (3-5), which not only has social and economic implications but also results in a loss of quality of life, functional limitations, frailty, and mortality (6,7). In the current context, developing strategies with a focus on treating aging as the common denominator of these conditions, rather than each specific disease, can be of tremendous relevance, extending health and delaying, preventing, or alleviating age-related disorders.

The cellular mechanisms involved in aging include telomere shortening, genomic instability, epigenetic alterations, mitochondrial dysfunction, loss of protein homeostasis (proteostasis), depletion and decline of stem cells, nutrient sensing deregulation, immune system decline, and cellular senescence (8,9). These processes are interrelated (10), linked and overlap. Senescence is not only a cause but also a consequence of several of them with senescent cells acting as true "synchronizers" of aging in different tissues, organs, or systems. Therefore, the objective of this work was to conduct a review in this rapidly growing field in recent years of undeniable translational interest.

CONCEPT

Cellular senescence is defined as a stable state of cell cycle arrest in response to different stimuli, in which cells cease proliferation and acquire an altered phenotype, thus losing their primary functionality. The concept was first described back in 1961 by Hayflick et al. (11) in human diploid fibroblasts cultured *in vitro*. It was demonstrated that normal cells have a limited capacity for division due to telomere shortening. This phenomenon

is now known as replicative senescence, although many other stress stimuli triggering cellular senescence have been discovered.

Overall, types of senescence can be grouped based on their triggers into replicative senescence, DNA damage-induced senescence, oncogene-induced senescence, oxidative stress-induced senescence, mitochondrial dysfunction-associated senescence, epigenetically induced senescence, paracrine senescence, and endocrine senescence (induced by the inflammatory environment generated by senescent cells themselves) (12). DNA damage such as telomere shortening and single- and double-strand breaks, oncogenic mutations (eg, Ras, Myc, B-Raf) that typically affect genes involved in cell cycle control, reactive metabolites like reactive oxygen species (ROS) and bioactive lipids, signals of elevated mitogens and nutrients that increase the mammalian target of rapamycin (mTOR) activity, proteotoxic stress such as protein aggregation and unfolded proteins, and damage-associated molecular patterns (DAMPs), among others, have been found to induce senescence. Most of these findings have been demonstrated in cell culture experiments and have been considered inducers of senescence *in vivo* ever since (13).

All these effectors contribute to widespread changes in gene expression, metabolism, and chromatin organization behind the growth arrest associated with senescence, structural changes in cells, and a specialized secretory activity known as the senescence-associated secretory phenotype (SASP). Additionally, it generates an inflammatory environment. Structural changes described in senescent cells include flattened, vacuolated, and enlarged morphology, altered composition of the plasma membrane, and accumulation of lysosomes and mitochondria (14).

PHYSIOLOGY AND PATHOPHYSIOLOGY OF SENESCENCE

The general objective of senescence is the elimination of unwanted cells in the body. Therefore, its involvement in cancer defense is vital: in response to oncogenic agents, senescence is induced, and replication of cancer cells is halted (the proapoptotic SASP

can even eliminate surrounding tumor cells) (3,10). Under physiological conditions, it plays a key role in the response to damage or stress and in tissue repair and remodeling, such as in wound healing processes (15). Its physiological role in embryonic development is well-established, as it participates in the formation of various anatomical structures during organogenesis and regulates the proportion of different cell types (complementing apoptosis). It also occurs in healthy adult tissues as a mechanism for the maturation of megakaryocytes and syncytiotrophoblasts in the placenta and for organism protection (12,13).

When senescent cells are not efficiently eliminated and accumulate, far from promoting regeneration, they exacerbate tissue dysfunction and contribute to the genesis of diseases (16,17). Senescence not only occurs with age but also drives aging by synchronizing it in different tissues and systems (18,19).

From the above, it can be deduced that cellular senescence is a complex phenomenon that can act as a *defense* mechanism to halt disease progression (3) under physiological conditions, while in other situations, it may promote *disease development*. When localized and limited in time, it promotes tissue remodeling during growth or after tissue damage, but it also contributes to the decline of the regenerative and functional potential of tissues, inflammation, and tumorigenesis when pronounced or persistent, as seen in aged organisms.

SIGNALING PATHWAYS OF SENESENCE

From a molecular perspective, cellular senescence can be understood as a cellular fate that occurs at any point in life and involves the action of external and internal inducers, transcription factor cascades, changes in gene expression, and chromatin remodeling (20). Initially, the activation of the p53/p21^{Cip1} and tumor suppressor p16^{INK4a} pathways occurs, generating a response that takes time to fully establish and is irreversible. The process is reinforced by an intracellular signaling loop that includes ROS (reactive oxygen species) linked to DNA damage responses, NFκB (nuclear factor kappa B), TGF-β

(transforming growth factor beta), and GATA4 (guanine adenine thymine adenine), as well as an IL-1 α , IL-6, and CCAAT-enhancer-binding protein beta (C/EBP- β) loop (5).

The senescent phenotype acquired by these cells is generally accompanied by an increased secretion of proinflammatory factors: TGF- β , NF κ B, IL-1 α , IL-6, IL-8, chemokines that draw and anchor immune cells, and activation of different enzymes such as metalloproteinases. Other characteristics of the senescent phenotype include nuclear expression of cell cycle inhibitors and tumor suppressors (p15, p16, p21, p27, p53, hypo-phosphorylated Rb), absence of proliferative markers like Ki67, expression of DNA damage markers, presence of senescence-associated heterochromatin foci (SAHF) in the nucleus, and accumulation of lipofuscin (20). As mentioned, senescent cells upregulate several antiapoptotic pathways known as senescent cell anti-apoptotic pathways (SCAPs): BCL-2/BCL-w/BCL-XL family pathway, PI3K (phosphatidylinositol-3-kinase)/Akt pathway, p53/p21/serpine pathway, ephrins/dependence receptor/tyrosine kinase pathway, HIF-1 α (hypoxia-inducible factor 1 alpha) pathway, and HSP-90 (heat shock protein 90) pathway (21, 22), as they need to resist apoptosis to protect themselves from their own proapoptotic SASP. These pathways represent a vulnerability of senescent cells and have paved the way for the identification of the first class of drugs capable of targeting them (senolytics), as described later. We should also mention that although the SASP is a characteristic feature, not all senescent cells develop it (10).

Identifying the senescent state is useful not only to locate these cells but, more importantly, to develop targeted therapies and assess their effects on senescent cells or the SASP (Fig. 1). In further investigations, the phenotypic characteristics and molecular biomarkers of senescence have been discovered both in cultured cells and tissues. However, these markers are nonspecific, and senescent cells are heterogeneous. Therefore, the approach to detect senescent cells *in vivo* currently involves the combination of multiple methods, as summarized on table I (13). It remains to be determined which methods would be most efficient for future clinical practice.

One of the classic markers of senescence, traditionally used *in vitro* in skeletal tissues such as skin or adipose tissue, is the increased activity of the lysosomal enzyme β -galactosidase or senescence-associated β -galactosidase (SA β Gal). The reason why this increased activity is detected in senescent cells is due to the high lysosomal content present in these cells (12).

ASSOCIATED DISEASES

Although senescent cells can appear at any stage of life, it is known that they accumulate in tissues as chronological age increases: adipose tissue, lung, skeletal muscle, heart, kidney, bone (13,17,19). As a matter of fact, the transplantation of small amounts of senescent cells around the knee joint in young mice induces a condition similar to osteoarthritis (23). Other conditions in which the accumulation of senescent cells has been demonstrated include progeroid syndromes in children, preeclampsia, age-related macular degeneration, liver cirrhosis, cancer, and vertebral spondylosis (4,10).

Similarly, recent studies have shown that senescent cells and the SASP play a prominent role in mediating age-related conditions such as cancer, osteoporosis, frailty, cardiovascular diseases, osteoarthritis, diabetes, and obesity, among others (3,6,16).

SENOLYTICS AND SENOMORPHICS

Given the potential of eliminating senescent cells or their proinflammatory secretion to treat age-related diseases and their consequences, the detection of senescent cells and the development of therapies targeting them have become important areas of research in the biomedical field. To this date, 2 main categories of drugs are being studied: *senolytics*, which specifically eliminate senescent cells, and *senomorphics*, which suppress the SASP without inducing cell death.

The first evidence supporting the hypothesis that treating senescent cells can alleviate age-related chronic diseases came from the creation of INK-ATTAC, a transgenic mouse model in which it is possible to identify (using p16^{Ink4a}), isolate, and selectively eliminate senescent cells by administering a synthetic activating molecule: AP20187 (24). It was demonstrated that the elimination of p16^{Ink4a}-positive senescent cells improved lifespan and had beneficial effects in multiple tissues (25).

Despite the promising nature of these findings, as it involves the insertion of a transgene, this genetic approach cannot be applied to humans (26). The first class of drugs that selectively eliminate senescent cells, *senolytics*, was then identified. These are small molecules that induce cell death by apoptosis specifically in senescent cells. They work by transiently disabling SCAPs, which, as mentioned earlier, protect senescent cells from their own proapoptotic SASP. The first senolytic compounds described, first *in vitro* and then *in vivo*, were dasatinib (D) and quercetin (Q), used together (D + Q). Dasatinib is a tyrosine kinase inhibitor, and quercetin is a flavonoid found in fruits and vegetables that inhibits tumor necrosis factor alpha (TNF α) (21).

In preclinical models, senolytics have been shown to delay, prevent, and/or alleviate frailty, cancer, and cardiovascular, hepatic, musculoskeletal, and neurological disorders. Initial trials suggest that they reduce senescent cell burden, decrease inflammation, and alleviate frailty in humans. Numerous clinical trials are currently underway for various diseases to safely translate these findings into the routine clinical practice in the management of age-related degenerative diseases (10,16).

We should mention that due to the beneficial functions of senescent cells, operating on the mechanisms through which a cell becomes senescent could have detrimental effects, such as an increased risk of cancer (27-30). Therefore, the goal is to target already formed senescent cells that accumulate damage and cause tissue dysfunction through their proinflammatory SASP. The following are the most important characteristics of each group.

As mentioned before, senescent cells resist apoptosis through their SCAPs. Senolytics act by transiently disabling these SCAPs, thus leading to cell death.

If we classify senolytic agents based on the anti-apoptotic pathways they target, as shown on figure 2, they can be divided into the following categories (16,22):

- BCL-2/BCL-W/BCL-XL pathway: Navitoclax, fisetin, A1331852, A1155463.
- PI3K/Akt pathway: quercetin, fisetin, piperlongumine.
- p53/p21/serpine pathway: quercetin, fisetin, FOXO4-related peptide.
- Ephrin/dependency receptor/tyrosine kinase pathway: dasatinib (ephrin receptor), piperlongumine (androgen receptors).
- HIF-1 α pathway: quercetin, fisetin.
- HSP-90 pathway: tanespimycin, alvespimycin (this pathway was discovered later) (31).

Recently, it has been discovered that mitoTAM (mitochondria-targeted tamoxifen) acts as a senolytic drug, and it is postulated to be part of a new group of agents targeting mitochondria, which is still not fully understood (16).

Among the first batch of senolytics discovered (10), using the aforementioned approach, we find the following ones: dasatinib, quercetin (21), fisetin (32,33), luteolin, curcumin, curcumin analog EF24, navitoclax (ABT263) (34,35), A1331852, A1155463, geldanamycin, tanespimycin, alvespimycin, piperlongumine (36), FOXO4-related peptide (37), nutlin3a, ouabain, and proscurcinidin. Some of them are natural compounds, while others are synthetic small molecules (22).

Currently, methods for identifying senolytic drugs include random screenings of drug libraries, the use of nanotechnology, or immunomodulators (10).

From the perspective of finding drugs that are as specific as possible, it is interesting that they can target multiple pathways, which reduces the chances of off-target effects. A specific case that illustrates this issue is Navitoclax (34,35), that acts on a restricted range of senescent cells but has apoptotic effects on non-senescent cells. Therefore, its clinical use is limited due to side effects such as severe thrombocytopenia and neutropenia (30).

The SCAPs necessary to survive apoptosis differ among different types of senescent cells, making it challenging to find a single senolytic that is effective against all of them (4,22,30).

Senolytics can act synergistically (4,22), as is the case with D + Q, thus opening up the possibility of expanding their spectrum of action by combining different molecules, similar to antibiotics.

Since senescent cells take time to accumulate again in a tissue and acquire a SASP, administering senolytic drugs intermittently may be enough to achieve the therapeutic goals intended, thus minimizing side effects and the risk of effects beyond the intended site of action, and allowing administration during periods of good health (21). Additionally, since senescent cells do not divide, it is unlikely that these drugs will generate resistance. Regarding dosage, it will likely depend on the circumstance leading to senescence, which will determine a specific accumulation rate for each process (4,22,38).

Prior to clinical trials in humans to demonstrate the effectiveness of a senolytic drug, it is important to make sure that the effects seen are due to its intended action and not to off-target effects. Modified Koch's postulates (4) have been proposed and are fulfilled in mice treated with D + Q for various diseases. There are also indications of their fulfillment for fisetin, although there are more doubts surrounding Navitoclax (10).

As mentioned earlier, regarding the treatment of senescence, there is another category of drugs called *senomorphic* or *senomodulating* agents, which are small molecules that act indirectly on senescent cells by inhibiting their inflammatory SASP.

Some of these agents include resveratrol, apigenin, kaempferol, metformin, glucocorticoids, rapamycin, everolimus, ruxolitinib, or EGCG (epigallocatechin gallate). Among them, some are natural compounds, while others are approved drugs for specific indications by regulatory agencies. However, it remains to be seen whether dosages would be similar. Additionally, there are molecules identified as potential senomorphic agents, such as loperamide (4,16).

The targets of these drugs are the pathways through which the SASP is expressed or acts (Fig. 3). The most important pathways, along with some notable agents that act on them, are (16):

- Inhibition of NFκB pathway: resveratrol, apigenin, kaempferol, metformin, glucocorticoids.
- Inhibition of mTOR pathway: rapamycin, everolimus.
- JAK (janus kinase)/STAT pathway (JAK inhibition): ruxolitinib.
- Antibodies against the activity and function of specific SASP mediators such as IL-6 or IL-8.

BONE SENESCENCE

The role of senescence in age-related bone loss has recently been the subject of numerous investigations. With aging, bone remodeling is disrupted, thus leading to an imbalance between the amount of new bone formation and bone resorption, resulting in a negative balance that, over time, leads to osteoporosis.

Aging manifests in the bone as a decrease in bone tissue itself and an increased marrow fat. It is not clear whether marrow fat has a direct negative effect on bone formation, although it is likely because mesenchymal stem cells (MSC) are precursors to both marrow adipocytes and osteoblasts, thus suggesting a shift towards the adipogenic lineage that, along with adipokines, may contribute to the described situation.

While the exact processes that cause these age-related changes are still not fully understood, it has been hypothesized that the basic mechanisms of aging including senescence, are responsible for age-related bone dysfunction (13).

For years, it has been impossible to demonstrate that senescent cells accumulate in bone with aging, as seen in other tissues. It was also unknown which cells in the bone microenvironment become senescent with age and whether they are capable of generating SASP (4). In the first study that addressed these issues (39), senescence and SASP markers were measured *in vivo* in young mice (both male and female) and compared to old mice, in highly enriched populations of various cell lineages. It was

discovered that the expression of p16^{Ink4a} consistently increased with aging in B cells, T cells, myeloid cells, osteoprogenitors, osteoblasts, and osteocytes. Additionally, it was found that p21^{Cip1} levels increased with aging in isolated cells from males enriched with osteocytes (while no change was seen in females). These findings in mice were validated in humans through the obtention of bone biopsies from elderly and young women, which revealed an age-related increase in both p16^{Ink4a} and p21^{Cip1}. Finally, it was demonstrated that SASP is mainly produced by senescent osteoprogenitors, senescent myeloid cells, and senescent osteocytes.

Although there are still significant gaps in knowledge and many unanswered questions, cellular senescence appears to be a global characteristic of natural aging in the bone, as in the rest of the body. This opens up the possibility that targeting senescence specifically could reduce the impact of a disease such as osteoporosis.

To determine if cellular senescence plays a role in age-related bone loss, 3 strategies were used: a genetic approach, a pharmacological approach to eliminate senescent cells (INKATTAC or senolytics, D + Q), and a senomorphic approach (using a JAK inhibitor, ruxolitinib) to inhibit SASP (26). All 3 interventions demonstrated prevention of bone loss in old mice, and none had effects on bone parameters in young mice, which is indicative of their specificity for aging (25).

In conclusion, studies on senescence in bone demonstrate that with aging, cells in the bone microenvironment (at least a subset of most cell types) become senescent and develop a heterogeneous SASP. Furthermore, they establish that senescent cells play a causal role in age-related bone loss, which can be alleviated in old mice by reducing the genetic or pharmacological burden with the first class of senolytics or with a senomorphic approach. This could represent a new strategy for treating or preventing osteoporosis, with potential advantages over conventional therapy (25,26).

Bisphosphonates are drugs commonly used in clinical practice to treat osteoporosis. One of them, zoledronic acid, could be considered a senotherapeutic agent. Animal experiments have shown that zoledronic acid extends cellular survival and delays senescence of mesenchymal stem cells, improves DNA repair by inhibiting the

mevalonate and mTOR pathways, improves intestinal epithelial dysplasia, and prevents radiation-induced mutations (41). In 2007, an effect on the reduction of mortality regardless of the effect on the rate of fracture was observed. Since then, clinical trials, meta-analyses, and observational studies specifically designed to measure this relationship suggest that bisphosphonates (particularly nitrogen-containing ones including zoledronic acid) provide survival benefits in osteoporotic/osteopenic patients, as well as in patients previously exposed to various circumstances in intensive care, with cancer, or with heart disease (43). Recently, in women in their seventh decade of life with osteopenia, the administration of 5mg of IV zoledronic acid every 18 months showed a clear trend towards lower rates of mortality, cancer, and cardiovascular events (44), indicative of a potential systemic senotherapeutic effect.

As a matter of fact, a review article on the effects of bisphosphonates and lifespan shows studies in which bisphosphonates exhibit a protective effect with hazard ratios ranging from 0.56 to 0.94 (43).

Although further studies are needed to confirm these effects, the results obtained so far are extremely promising in the sense that drugs already used and useful in the treatment of a prevalent disease like osteoporosis, such as zoledronic acid, may have additional benefits similar to those described. Furthermore, understanding this association of zoledronic acid with increased survival opens the door to investigating the behavior of senescent cell markers in patients receiving zoledronic acid treatment and patients from other therapeutic groups, to identify surrogate markers of survival that would simplify future studies in this field.

CONCLUSIONS

Cellular senescence is a phenomenon involved in aging and chronic diseases associated with aging including osteoporosis. Its mechanism is complex and ambivalent: while it can be beneficial in certain contexts, especially in young tissues, it can be detrimental due to accumulation and its senescence-associated secretory phenotype (SASP) in other cases, leading to tissue dysfunction or exacerbating it.

To mitigate its role in the development of age-related diseases, therapies targeting senescent cells or their SASP are being investigated. A more comprehensive study of senescence markers would allow us to study the effect of certain drugs already used clinically for other indications and with lower risk compared to novel drugs.

ACKNOWLEDGEMENTS

The authors would like to thank the support given by Instituto de Salud Carlos III RICORS2040 (Renal Disease), the European Regional Development Fund (ERDF), and the Science, Technology, and Innovation Plan 2013-2017 and 2018-2022 of the Principality of Asturias, Spain (IDI-2021-000080).

REFERENCES

1. Seals DR, Justice JN, LaRocca TJ. Physiological geroscience: targeting function to increase healthspan and achieve optimal longevity. *J Physiol* 2016;594(8):2001-2024. DOI: 10.1113/jphysiol.2014.282665
2. United Nations. Envejecimiento [Internet]. Naciones Unidas. 2019 [citado 20 de marzo de 2022]. Disponible en: <https://www.un.org/es/global-issues/ageing>
3. Song S, Lam EW, Tchkonja T, Kirkland JL, Sun Y. Senescent Cells: Emerging Targets for Human Aging and Age-Related Diseases. *Trends Biochem Sci* 2020;45(7):578-92. DOI: 10.1016/j.tibs.2020.03.008
4. Khosla S, Farr JN, Tchkonja T, Kirkland JL. The role of cellular senescence in ageing and endocrine disease. *Nat Rev Endocrinol* 2020;16(5):263-75. DOI: 10.1038/s41574-020-0335-y
5. Tchkonja T, Zhu Y, van Deursen J, Campisi J, Kirkland JL. Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. *J Clin Invest* 2013;123(3):966-72. DOI: 10.1172/JCI64098

6. Kaur J, Farr JN. Cellular senescence in age-related disorders. *Transl Res* 2020;226:96104. DOI: 10.1016/j.trsl.2020.06.007
7. Kirkland JL, Stout MB, Sierra F. Resilience in Aging Mice. *J Gerontol A Biol Sci Med Sci* 2016;71(11):1407-14. DOI: 10.1093/gerona/glw086
8. Kennedy BK, Berger SL, Brunet A, Campisi J, Cuervo AM, Epel ES, et al. Geroscience: linking aging to chronic disease. *Cell* 2014;159(4):709-13. DOI: 10.1016/j.cell.2014.10.039
9. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell* 2013;153(6):1194-217. DOI: 10.1016/j.cell.2013.05.039
10. Kirkland JL, Tchkonian T. Senolytic drugs: from discovery to translation. *J Intern Med* 2020;288(5):518-36. DOI: 10.1111/joim.13141
11. Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. *Exp Cell Res* 1961;25:585-621. DOI: 10.1016/0014-4827(61)90192-6
12. Galiana Guillem I, Martínez Máñez R (dir), Orzáez Calatayud M (dir). Desarrollo de nuevos nanodispositivos terapéuticos aplicados al tratamiento de enfermedades relacionadas con procesos de senescencia [tesis doctoral en Internet]. [Valencia]: Universitat Politècnica de València; 2020 [citado 15 marzo de 2022]. Disponible en: <http://hdl.handle.net/10251/151950>
13. Farr JN, Khosla S. Cellular senescence in bone. *Bone*. 2019;121:121-33. DOI: 10.1016/j.bone.2019.01.015
14. Hernandez-Segura A, Nehme J, Demaria M. Hallmarks of Cellular Senescence. *Trends Cell Biol* 2018;28(6):436-53. DOI: 10.1016/j.tcb.2018.02.001
15. Demaria M, Ohtani N, Youssef SA, Rodier F, Toussaint W, Mitchell JR, et al. An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. *Dev Cell* 2014;31(6):722-33. DOI: 10.1016/j.devcel.2014.11.012
16. Lagoumtzi SM, Chondrogianni N. Senolytics and senomorphics: Natural and synthetic therapeutics in the treatment of aging and chronic diseases. *Free Radic Biol Med* 2021;171:169-90. DOI: 10.1016/j.freeradbiomed.2021.05.003

17. Tchkonina T, Palmer AK, Kirkland JL. New Horizons: Novel Approaches to Enhance Healthspan Through Targeting Cellular Senescence and Related Aging Mechanisms. *J Clin Endocrinol Metab* 2021;106(3):e1481-e1487. DOI: 10.1210/clinem/dgaa728
18. DeVito LM, Barzilai N, Cuervo AM, Niedernhofer LJ, Milman S, Levine M, et al. Extending human healthspan and longevity: a symposium report. *Ann N Y Acad Sci* 2022;1507(1):70-83. DOI: 10.1111/nyas.14681
19. Kirkland JL, Tchkonina T. Cellular Senescence: A Translational Perspective. *EBioMedicine* 2017;21:21-8. DOI: 10.1016/j.ebiom.2017.04.013
20. Gorgoulis V, Adams PD, Alimonti A, Bennett DC, Bischof O, Bishop C, et al. Cellular Senescence: Defining a Path Forward. *Cell* 2019;179(4):813-27. DOI: 10.1016/j.cell.2019.10.005
21. Zhu Y, Tchkonina T, Pirtskhalava T, Gower AC, Ding H, Giorgadze N, et al. The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. *Aging Cell* 2015;14(4):644-58. DOI: 10.1111/ace.12344
22. Kirkland JL, Tchkonina T, Zhu Y, Niedernhofer LJ, Robbins PD. The Clinical Potential of Senolytic Drugs. *J Am Geriatr Soc* 2017;65(10):2297-301. DOI: 10.1111/jgs.14969
23. Xu M, Bradley EW, Weivoda MM, Hwang SM, Pirtskhalava T, Decklever T, et al. Transplanted Senescent Cells Induce an Osteoarthritis-Like Condition in Mice. *J Gerontol A Biol Sci Med Sci* 2017;72(6):780-5.
24. Baker DJ, Wijshake T, Tchkonina T, LeBrasseur NK, Childs BG, van de Sluis B, et al. Clearance of p16Ink4a-positive senescent cell delays ageing-associated disorders. *Nature* 2011;479(7372):232-6. DOI: 10.1038/nature10600
25. Khosla S, Farr JN, Kirkland JL. Inhibiting Cellular Senescence: A New Therapeutic Paradigm for Age-Related Osteoporosis. *J Clin Endocrinol Metab* 2018;103(4):1282-1290. DOI: 10.1210/jc.2017-02694

26. Farr JN, Xu M, Weivoda MM, Monroe DG, Fraser DG, Onken JL, et al. Targeting cellular senescence prevents age-related bone loss in mice. *Nat Med* 2017;23(9):1072-9. DOI: 10.1038/nm.4385
27. Takeuchi S, Takahashi A, Motoi N, Yoshimoto S, Tajima T, Yamakoshi K, et al. Intrinsic cooperation between p16Ink4a and p21Waf1/Cip1 in the onset of cellular senescence and tumor suppression in vivo. *Cancer Res* 2010;70(22):9381-90. DOI: 10.1158/0008-5472.CAN-10-0801
28. Larsen CJ. PRB, p53, p16Ink4a/ARF, sénescence cellulaire et transformation maligne [pRB, p53, p16Ink4a, senescence and malignant transformation]. *Bull Cancer* 2004;91(5):399-402. French.
29. Faget DV, Ren Q, Stewart SA. Unmasking senescence: context-dependent effects of SASP in cancer. *Nat Rev Cancer* 2019;19(8):439-53. DOI: 10.1038/s41568-019-0156-2
30. Kang C. Senolytics and Senostatics: A Two-Pronged Approach to Target Cellular Senescence for Delaying Aging and Age-Related Diseases. *Mol Cells* 2019;42(12):821827.
31. Fuhrmann-Stroissnigg H, Ling YY, Zhao J, McGowan SJ, Zhu Y, Brooks RW, et al. Identification of HSP90 inhibitors as a novel class of senolytics. *Nat Commun* 2017;8(1):422. DOI: 10.1038/s41467-017-00314-z
32. Zhu Y, Doornebal EJ, Pirtskhalava T, Giorgadze N, Wentworth M, Fuhrmann-Stroissnigg H, et al. New agents that target senescent cells: the flavone, fisetin, and the BCL-XL inhibitors, A1331852 and A1155463. *Aging (Albany NY)* 2017;9(3):955-63. DOI: 10.18632/aging.101202
33. Yousefzadeh MJ, Zhu Y, McGowan SJ, Angelini L, Fuhrmann-Stroissnigg H, Xu M, et al. Fisetin is a serotherapeutic that extends health and lifespan. *EBioMedicine* 2018;36:18-28. DOI: 10.1016/j.ebiom.2018.09.015
34. Zhu Y, Tchkonja T, Fuhrmann-Stroissnigg H, Dai HM, Ling YY, Stout MB, et al. Identification of a novel senolytic agent, navitoclax, targeting the Bcl-2 family of antiapoptotic factors. *Aging Cell* 2016;15(3):428-35. DOI: 10.1111/accel.12445

35. Chang J, Wang Y, Shao L, Laberge RM, Demaria M, Campisi J, et al. Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. *Nat Med* 2016;22(1):78-83. DOI: 10.1038/nm.4010
36. Wang Y, Chang J, Liu X, Zhang X, Zhang S, Zhang X, et al. Discovery of piperlongumine as a potential novel lead for the development of senolytic agents. *Aging (Albany NY)* 2016;8(11):2915-26. DOI: 10.18632/aging.101100
37. Baar MP, Brandt R, Putavet DA, Klein J, Derks K, Bourgeois B, et al. Targeted Apoptosis of Senescent Cells Restores Tissue Homeostasis in Response to Chemotoxicity and Aging. *Cell* 2017;169(1):132-147.e16. DOI: 10.1016/j.cell.2017.02.031
38. Palmer AK, Gustafson B, Kirkland JL, Smith U. Cellular senescence: at the nexus between ageing and diabetes. *Diabetología* 2019;62(10):1835-41. DOI: 10.1007/s00125-019-4934-x
39. Farr JN, Fraser DG, Wang H, Jaehn K, Ogrodnik MB, Weivoda MM, et al. Identification of Senescent Cells in the Bone Microenvironment. *J Bone Miner Res* 2016;31(11):1920-1929. DOI: 10.1002/jbmr.2892
40. Xu M, Tchkonja T, Ding H, Ogrodnik M, Lubbers ER, Pirtskhalava T, et al. JAK inhibition alleviates the cellular senescence-associated secretory phenotype and frailty in old age. *Proc Natl Acad Sci U S A* 2015;112(46):E6301-E6310. DOI: 10.1073/pnas.1515386112
41. Misra J, Mohanty ST, Madan S, Fernandes JA, Hal Ebetino F, Russell RG, et al. Zoledronate Attenuates Accumulation of DNA Damage in Mesenchymal Stem Cells and Protects Their Function. *Stem Cells* 2016;34(3):756-67. DOI: 10.1002/stem.2255
42. Lyles KW, Colón-Emeric CS, Magaziner JS, Adachi JD, Pieper CF, Mautalen C, et al; HORIZON Recurrent Fracture Trial. Zoledronic acid and clinical fractures and mortality after hip fracture. *N Engl J Med* 2007;357(18):1799-809. DOI: 10.1056/NEJMoa074941

43. Center JR, Lyles KW, Bliuc D. Bisphosphonates, and lifespan. *Bone* 2020;141:115566. DOI: 10.1016/j.bone.2020.115566
44. Reid IR, Horne AM, Milhov B, Stewart A, Garratt E, Bastin S, et al. Effects of Zoledronate on Cancer, Cardiac Events, and Mortality in Osteopenic Older Women. *J Bone Miner Res* 2020;35(1):20-7. DOI: 10.1002/jbmr.3860
45. Marie PJ. Bone cell senescence: mechanisms and perspectives. *J Bone Miner Res* 2014;29(6):1311-21. DOI: 10.1002/jbmr.2190
46. Marie PJ, Cohen-Solal M. The Expanding Life and Functions of Osteogenic Cells: From Simple Bone-Making Cells to Multifunctional Cells and Beyond. *J Bone Miner Res* 2018;33(2):199-210. DOI: 10.1002/jbmr.3356
47. Reid IR, Horne AM, Mihov B, Stewart A, Garratt E, Wong S, et al. Fracture Prevention with Zoledronate in Older Women with Osteopenia. *N Engl J Med* 2018;379(25):2407-2416. DOI: 10.1056/NEJMoa1808082
48. Xu M, Pirtskhalava T, Farr JN, Weigand BM, Palmer AK, Weivoda MM, et al. Senolytics improve physical function and increase lifespan in old age. *Nat Med* 2018;24(8):1246-1256. DOI: 10.1038/s41591-018-0092-9
49. González-Gualda E, Baker A G, Fruk L, Muñoz-Espín D. A guide to assessing cellular senescence in vitro and in vivo. *FEBS J* 2021;288(2):56-80. DOI: 10.1111/febs.15570
50. Sun J, Li Y, Yang X, Dong W, Yang J, Hu Q, et al. Growth differentiation factor 11 accelerates liver senescence through the inhibition of autophagy. *Aging Cell* 2022;21:e13532. DOI: 10.1111/accel.13532

Table I. Cellular senescence signaling at different levels

Senescence promoters	Senescence effector genes	p16 ^{Ink4a} , p21 ^{Cipl} , and transgenes such as p16-LUC, INK-ATTAC [EGFP, FLAG], 3MR [mRFP] p16-LUC, INK-ATTAC [EGFP, FLAG], 3MR [mRFP]
	DNA damage	γH2AX, TAFs (co-localization of DNA damage with telomeric repeat sequences), phosphorylated p53
	Cell cycle arrest	p16 ^{Ink4a} , p21 ^{Cipl} , DNA synthesis rate
Regulators of the senescence process	Biomarkers of inflammatory environment (SASP)	IL-6, IL-8, IL1-α, IL1-Jβ, MCP-1, Pai-1, Pai-2, MMPs, Activin A, TNFα, TGFβ, NFκβ, CEBPβ, GATA4...
	Anti-apoptosis biomarkers	SCAPs, Bcl-2, Bcl-xL, Bcl-w...
	Autophagy modulation	GDF 11 (growth differentiation factor 11)
Histological alterations	Lysosomal dysfunction	Quantity and activity of lysosomal β-galactosidase at pH 6.0
	Mitochondrial accumulation	Mitotracker staining and morphology (fusion/fission)

	Morphological alterations	Enlargement, flattening, granularity, karyomegaly, heterochromatinization, chromosomal segregation failure, elevated CCFs
	Other biomarkers	Presence of lipofuscin (GL13 staining); loss of HMGB1 and decreased laminin B1

Bcl: B-cell Lymphoma; CCFs: cytoplasmic chromatin fragments; CEBP: CCAAT/enhancer-binding protein; GATA: guanine adenine thymine adenine; HMGB1: high mobility group box 1; H2AX: histone family member X; IL: interleukin; MCP1: monocyte chemoattractant protein-1; MMP: matrix metalloproteinase; NFκB: nuclear factor kappa B; SASP: senescence-associated secretory phenotype; SCAPs: anti-apoptotic pathways of senescent cells; TNF: tumor necrosis factor; Modified from: Farr JN, et al. (13,49,50).

Figure 1. Inducers and mediators of senescence, SCAPs (anti-apoptotic pathways of senescent cells), SASP (senescence-associated secretory phenotype), and therapeutic targets of senotherapeutics on senescent cells. Modified from: Farr JN, et al. Bone 2019 (13).

Figure 2. Main pathways targeted by senolytics. These include the BCL-2 family pathway, PI3K/Akt pathway, p53/p21/serpine pathway, ephrin/dependence receptor/tyrosine kinase pathway, and HIF-1α pathway. Modified from Lagoumtzi, et al. Free Radic Biol Med 2021 (16).

Figure 3. Main pathways targeted by senomorphics. These include the NFκB inhibition pathway, mTOR inhibition pathway, JAK/STAT pathway, and antibodies against specific SASP mediators. The mTOR kinase is key in cellular metabolism, controlling cellular

catabolism and anabolism, determining whether cells (particularly cancer cells) should grow and proliferate. Additionally, mTOR has effects on apoptosis regulation. NFκB is widely used by eukaryotic cells as a regulator of genes that control cell proliferation and survival. The JAK/STAT signaling pathway is involved in processes such as immunity, cell division, cell death, and tumor formation. Modified from Lagoumtzi, et al. Free Radic Biol Med 2021 (16).

TRADUCCIÓN DE FIGURAS

(Color negro: español · Color azul: inglés)

Figura 1

Daño en el ADN

Mutaciones oncogénicas

Metabolitos reactivos

Mitógenos/IGF-1

Acortamiento de telómeros

Estrés epigenético

Estrés proteotóxico

DAMPs

DNA damage

Oncogenic mutations

Reactive metabolites

Mitogens/IGF-1

Telomere shortening

Epigenetic stress

Proteotoxic stress

DAMPs

Activación de genes

[Gene activation](#)

P16/Rb

[P16/Rb](#)

Activación de genes

[Gene activation](#)

p53/p21

[p53/p21](#)

Senolíticos

[Senolytics](#)

SCAPs

[SCAPs](#)

Familia BCL-2

[BCL-2 family](#)

P13K/Akt

[PI3K/Akt](#)

p53/p21

[p53/p21](#)

Tirosin Kinasa

[Tyrosine kinase](#)

HIF-1 alfa

[HIF-1 alpha](#)

HSP-90

[HSP-90](#)

SENESCENCIA

[SENESCENCE](#)

IL-1a

IL-6 > GATA4/TGFb/NFKB

C/EBPb

[IL-1a](#)

[IL-6 > GATA4/TGFb/NFκB](#)

[C/EBPb](#)

SASP

[SASP](#)

Especies reactivas de oxígeno

[Reactive oxygen species](#)

Disfunción mitocondrial

[Mitochondrial dysfunction](#)

Resistencia a la apoptosis

Apoptosis resistance

Senomórficos

Senomorphics

SASP

SASP

Disfunción tisular

Tissue dysfunction

Fragilidad, enfermedades crónicas, envejecimiento

Fragility, chronic diseases, aging

Figura 2

Inhibidores JAK

(ruxolitinib)

JAK inhibitors

(ruxolitinib)

Inhibidores mTOR

(rapamicina, everolimus)

mTOR inhibitors

(rapamycin, everolimus)

JAK/STAT

mTOR

JAK/STAT

mTOR

Daño inducido por el SASP

SASP-induced damage

Genes SASP

SASP genes

YYY

YYY

Posibles resultados

Supresión del SASP

Actividad Bgalactosidasa

P21, p16

Inflamación

Fenotipo “más joven”

Potential outcomes

Suppression of SASP

Beta-galactosidase activity

P21, p16

Inflammation

"More youthful" phenotype

Inhibidores NFkB

(resveratrol, apigenina, kaempferol, metformina, corticoids)

NFkB inhibitors

(resveratrol, apigenin, kaempferol, metformin, corticosteroids)

Anticuerpos contra factores SASP específicos

(neutralización de actividad y función)

Antibodies against specific SASP factors

(neutralization of activity and function)

Figura 3

AGOTAMIENTO REPLICATIVO

REPLICATIVE EXHAUSTION

SENESCENCIA CELULAR

CELLULAR SENESENCE

FACTORES ESTRESANTES:

Estrés oxidativo

Radiación UV

Quimioterapia

Estrés genotóxico

Señalización oncogénica

STRESS FACTORS:

Oxidative stress

UV radiation

Chemotherapy

Genotoxic stress

Oncogenic signaling

Disfunción mitocondrial e incremento de ROS*

Acumulación de lipofuscina

Foci dañado del ADN

SASP

Foci de heterocromatina de ADN

Mitocondria

[Mitochondrial dysfunction and increased ROS*](#)

[Accumulation of lipofuscin](#)

[DNA damaged foci](#)

[SASP](#)

[DNA heterochromatin foci](#)

[Mitochondria](#)

APOPTOSIS

[APOPTOSIS](#)

RESISTENCIA A LA APOPTOSIS

[RESISTANCE TO APOPTOSIS](#)

P13K/Akt

p53/MDM2

p53/p21/serpina

p53/FOXO4

HSP-90

NF-kB

Nrf2

HIF-1alfa

Acetilación de histonas

Enzimas redox-dependientes

Bomba Na⁺/K⁺ATPasa

[PI3K/Akt](#)

[p53/MDM2](#)

[p53/p21/serpine](#)

[p53/FOXO4](#)

[HSP-90](#)

[NF-kB Nrf2](#)

[HIF-1α](#)

[Histone acetylation](#)

[Redox-dependent enzymes](#)

[Na⁺/K⁺ ATPase pump](#)

Objetivo de los SCAPs

[Target of SCAPs](#)

Agentes senolíticos dirigidos a las mitocondrias: mitoTAM

[Mitochondria-targeted senolytics: mitoTAM](#)

BCL-2

BCL-xL

BCL-w

[BCL-2](#)

[BCL-xL](#)

[BCL-w](#)

Senolíticos inhibidores de la familia BCL-2 miméticos de BH3:

[BH3-mimetic BCL-2 family inhibitor senolytics:](#)

ABT-263 (Navitoclax)

ABT-737

A1331852

A1155463

UBX1325

[ABT-263 \(Navitoclax\)](#)

[ABT-737](#)

[A1331852](#)

[A1155463](#)

[UBX1325](#)

*ROS: especies reactivas de oxígeno (iones de oxígeno, radicales libres, peróxidos)

[*ROS, reactive oxygen species \(oxygen ions, free radicals, peroxides\)](#)

SENOLÍTICOS

[SENOLYTICS](#)

Compuestos naturales: quercitina, fisetina, piperlongumina, curcuma, vainillina, ouabaína, análogo de la curcuma EF24, digoxina.

[Natural compounds: quercetin, fisetin, piperlongumine, curcumin, vanillin, ouabain, EF24 curcumin analogue, digoxin.](#)

Inhibidores de la kinasa: dasatinib.

[Kinase inhibitors: dasatinib.](#)

Inhibidores de la interacción MDM2/p53: UBX0101, P5091.

[MDM2/p53 interaction inhibitors: UBX0101, P5091.](#)

Inhibidores HSP-90: geldanamicina, tanespimicina (17-AAG), alvespimicina (17-DMAG).

[HSP-90 inhibitors: geldanamycin, tanespimycin \(17-AAG\), alvespimycin \(17-DMAG\).](#)

Inhibidores de la union de p53: FOXO4-DRI.

[p53 binding inhibitors: FOXO4-DRI.](#)

Inhibidores HDAC: panobinostat.

[HDAC inhibitors: panobinostat.](#)