Senolytics: Potential for Alleviating Diabetes and Its Complications

Allyson K. Palmer, M.D., Ph.D.¹, Tamar Tchkonia, Ph.D.¹, James L. Kirkland, M.D., Ph.D.¹*

¹Robert and Arlene Kogod Center on Aging,
Mayo Clinic
200 1st Street SW,
Rochester, MN 55905

*To whom correspondence and reprint requests should be addressed at:
Robert and Arlene Kogod Center on Aging,
Mayo Clinic,
200 First Street, S.W.,
Rochester, MN 55905
Telephone: 507 266 9151

Grants supporting the writing of this paper: NIH grants R37AG013925, P01AG062413, R01AG 072301, R01DK120292, R33AG061456

Disclosure summary: JLK, AKP, and TT have a financial interest related to this research. Patents on senolytic drugs are held by Mayo Clinic. This research has been reviewed by the Mayo Clinic Conflict of Interest Review Board and was conducted in compliance with Mayo Clinic Conflict of Interest policies.
Abstract

Therapeutics that target cellular senescence, including novel “senolytic” compounds, hold significant promise for treating or preventing obesity-induced metabolic dysfunction, type 2 diabetes, and the multiple complications of diabetes and obesity. Senolytics selectively clear senescent cells, which accumulate with aging and obesity and represent a fundamental mechanism of aging that contributes to metabolic dysfunction and diabetes pathogenesis. In addition to improving metabolic function, targeting senescent cells holds promise as a preventative strategy to reduce incidence and severity of diabetes complications. The intermittent administration schedule utilized for senolytic therapy may confer benefits in terms of improving adherence and limiting adverse effects. It is necessary to design effective clinical trials that will safely translate discoveries from preclinical models into human studies that may pave the way for a novel therapeutic class for treating obesity, diabetes, and their complications. In this review, we outline what is known regarding the role of cellular senescence in the pathogenesis of type 2 diabetes and its complications, present evidence from preclinical models that targeting cellular senescence is beneficial, review senolytic drugs, and outline the features of clinical trials investigating the role of targeting senescent cells for diabetes.

Keywords: aging, cellular senescence, diabetes mellitus, diabetes complications
Introduction

Targeting fundamental aging mechanisms such as cellular senescence has broad potential to prevent, treat, or alleviate multiple age-related conditions simultaneously, rather than individually. This concept is termed the Geoscience Hypothesis. Diabetes itself is a major risk factor for premature development of age-related conditions, including cognitive impairment, cardiovascular disease, renal dysfunction, and others. Current therapeutic options focus mainly on glycemic control as a preventative measure and do not target end organ damage once present. Therefore, novel therapeutics are needed to target diabetes and its complications. Senescent cells accumulate with obesity, atherosclerosis, and aging. Senescent cells, which appear to play a role in development of diabetes and its associated conditions, are a promising therapeutic target based on mounting preclinical data. Senolytic, or senescence targeting, drugs are under development and are being investigated in early clinical trials across multiple diseases. Senolytics hold particular promise for obesity related metabolic dysfunction and diabetes. Well-designed trials are needed to investigate senolytic agents in diabetes and its complications.

Cellular Senescence in Obesity, Aging, and Diabetes: Features and Consequences

Cellular senescence is a cell fate that entails cell cycle arrest and occurs in response to a variety of stressors including metabolic signals (including hyperglycemia, saturated lipids, and reactive oxygen species), DNA damage, oncogene activation, and telomere shortening. Some senescent cells become enlarged, in part due to increased protein synthesis, and undergo changes in gene expression to produce the senescence-associated secretory phenotype, or SASP, which includes inflammatory factors, matrix remodeling enzymes, chemokines, cytokines, bioactive lipids (prostenoids, bradykines, ceramides), and nucleotides (including microRNAs and immune-modulatory mitochondrial DNA) [1-3]. Aging and
obesity, the major risk factors for type 2 diabetes, are both associated with increased burden of senescent cells, which accumulate in many tissues of the body [4-6]. Despite representing a small percentage of cells in any particular tissue, senescent cells have been implicated in a wide variety of diseases and organ dysfunctions, as well as reduced physical activity and early mortality [7-9].

Cellular senescence is thought to play a role in development of insulin resistance and diabetic complications, however the diabetic state can also cause formation of additional senescent cells, for example, through exposure to high glucose or lipid levels, which can themselves cause cellular senescence [10, 11]. Once senescent cells form, the SASP can spread senescence in a paracrine or endocrine manner to previously normal local or distant cells [2, 9, 12-14]. Senescent cells possess the ability to evade apoptosis through upregulation of anti-apoptotic pathways, including the PI3K/AKT, p53/p21^{CIP1}/serpine, HIF-1α, and BCL-2/BCL-XL pathways[15]. Reliance on these pathways, termed senescent cell anti-apoptotic pathways, or SCAPs, provides senescent cells with the ability to resist the pro-apoptotic and tissue-damaging factors that are components of their own SASP [15, 16]. Through these mechanisms, a pathogenic loop is formed that begets more cellular senescence. According to our ‘Threshold Theory of Cellular Senescence’, when the senescent cell population surpasses the immune system’s ability to clear them, senescent cells accumulate more rapidly and cause increased pathology [17]. Consistent with this theory, while transplantation of relatively few senescent cells into healthy younger animals induces early onset of age-related disease and frailty, a smaller number of cells is needed to exert this effect in aged animals, on top of their higher pre-existing burden of senescent cells [9, 18]. Obesity, like aging, is associated with senescent cell accumulation and therefore may lower the threshold of additional senescent cells needed to cause systemic dysfunction.
Adipose tissue can constitute a significant reservoir of senescent cells, particularly in obesity and diabetes [4, 5, 18]. Visceral adipose tissue contains the highest burden of senescent cells in diet-induced obese mice, and tends to be the location to which senescent cells migrate when transplanted intraperitoneally into experimental animals [9]. Senescent cell burden correlates with adipose cell size, even in non-diabetic individuals, and may be increased even before the development of type 2 diabetes in humans with a genetic predisposition to diabetes [4, 18, 19]. Polymorphisms in markers of cellular senescence, for example CDKN2A (which encodes p16^{INK2A}), confer increased risk of developing type 2 diabetes and cardiovascular disease [20]. Similarly, individuals with some progeroid syndromes such as those associated with Lamin-A (LMNA) mutations, which involve increased senescent cell burden, are also associated with lipodystrophy, metabolic syndrome, and diabetes [21, 22]. The tumor suppressor p53 is associated with cellular senescence and is upregulated in adipose tissue in obesity, type 2 diabetes, and aging [5, 23, 24]. p53 plays an important role in suppressing normal adipogenesis in obesity and blunts insulin-dependent glucose transport and induction of lipolysis [25]. These actions contribute to inflammation and insulin resistance. Cellular senescence also limits adipogenesis in a cell-autonomous manner, as well as through paracrine and endocrine effects of SASP factors such as activin A, TNF-α, and IL-6 [26-28]. Reduced adipogenesis limits adipose tissue capacity to store excess lipids and likely contributes to increased ectopic lipid deposition in other tissues, which can promote insulin resistance [29]. Additionally, senescent cells appear to play a role in the chemo-attraction of macrophages into visceral adipose tissue, which is associated with insulin resistance [18].
Senescent cell accumulation in other metabolic organs, such as muscle and liver, likely also plays a role in the development of insulin resistance [18, 30]. In the pancreas, genes related to cellular senescence, such as p16<sup>Ink4a</sup>, are increased with aging, and this is associated with reduced β-cell proliferative capacity. However, rather than having reduced insulin secretion, p16<sup>Ink4a</sup> positive β-cells actually were found to secrete more insulin, consistent with the observation that aged animals have increased basal insulin secretion [31]. Other studies have shown that clearance of senescent β-cells in a mouse model of type 1 diabetes actually improves insulin secretion, an effect that may be more relevant to the severe insulin deficiency seen in type 1 diabetes [32]. More work is needed to further elucidate the relationship between cellular senescence and insulin secretion by the pancreas.

**Senolytics: Drugs that Target Senescent Cells**

In 2004, caloric restriction, an intervention that extends life- and healthspan and delays age-related pathologies in mice, was found to be linked to delayed age-related senescent cell accumulation, indicating that senescent cell burden and healthspan are associated [33]. This prompted efforts to identify pharmacologic agents that target senescent cells to reduce the burden of age-related disorders and diseases [17, 34]. Successful identification of senolytics began with the discovery of senescent cell anti-apoptotic pathways, or SCAPs. Senescent cells were previously known to be resistant to apoptosis [35]. The necessity of each SCAP for senescent cell survival was tested using RNA interference, comparing the survival of senescent vs. non-senescent cells after knockdown of key nodes on different SCAPs. Drugs were identified by bioinformatics approaches that target these key SCAP nodes identified by RNA interference [15]. Using this mechanism-based approach, the first senolytics discovered were dasatinib (D) and quercetin (Q), first published in early 2015 [15]. These drugs were found to preferentially cause apoptosis in senescent cells without
significant effects on proliferating or quiescent cells. D is a tyrosine kinase inhibitor used for treating cancers, but unlike most other tyrosine kinase inhibitors, it inhibits Src kinases [36]. Q is a natural flavonoid and an inhibitor of PI3K, other kinases, and serpines [37, 38]. Subsequently, additional senolytic drugs including navitoclax (ABT263) [16, 39], and fisetin [40] were discovered. Senolytic drugs have differential effects on various senescent cell types. For example, D is relatively selective for senescent human adipose progenitor cells, whereas Q causes apoptosis of senescent human endothelial cells. Therefore, combinations of senolytic drugs (such as D+Q) target a broader range of senescent cell types than single agents, especially agents that have a single molecular target, which tend to target only a restricted range of senescent cells and may have more side effects than agents or combinations that act across SCAP networks [17, 34, 41, 42].

Senolytics do not prevent formation of senescent cells. This is important because generation of senescent cells is in large part an anti-cancer mechanism, limiting the ability of pre-cancerous or cancerous cells from dividing further. In addition, senescent cells have some beneficial effects, including in early wound healing [43]. Therefore, it would not be prudent to develop therapies that inhibit the ability of cells to become senescent. Therapies to target cellular senescence must act once senescent cells are already formed, particularly that 30 to 70% of senescent cells that have a tissue destructive SASP. This can be achieved by causing their removal with senolytics or limiting their effects on other cells and tissues, such as by inhibiting their SASP [2, 44].

Since senescent cells can take weeks to develop and acquire a SASP, and because only a brief exposure of senescent cells to senolytics is sufficient to cause them to be removed by apoptosis, administration of senolytic drugs can be in a “hit and run” fashion,
much like antibiotics or chemotherapeutics. In effect, senescent cells can be cleared in waves during each round of senolytic therapy. This may be superior to approaches that only mitigate the effects of senescent cells, for example SASP inhibitors, as SASP inhibitors and related agents need to be administered continuously. The effect of senolytics is durable because senescent cells undergo apoptosis in response to senolytic therapy and are therefore removed from tissue. As an example of the durability of response to senolytic therapy, positive effects of one round of senolytic treatment on the mobility of mice that had undergone radiation of one hind leg lasted for at least 7 months, despite the elimination half-life of each drug being only a few hours [15, 45-47]. The rate of accumulation of senescent cells will likely determine the optimal administration schedule of senolytics for any particular indication. For example, one course of senolytic treatment may be sufficient for dysfunction caused by senescent cells induced by therapeutic radiation or chemotherapy, however more frequent courses of treatment may be needed in diabetes due to ongoing metabolic insults causing ongoing formation of new senescent cells.

Like all drugs, senolytics are expected to have side effects. These are well characterized for some senolytic drugs, such as dasatinib and quercetin, which are both approved for use in humans. Dasatinib is used as chemotherapy continuously, in some cases for years, with a tolerable side effect profile. Pleural effusions and pulmonary hypertension can be seen with dasatinib therapy and are typically reversible upon discontinuation of the drug or preventable with dose reduction [48]. Quercetin has been used as a dietary supplement with rare side effects however has ability to cause nephrotoxicity at higher doses or in combination with other nephrotoxic agents [49]. Navitoclax, another senolytic drug, can cause transient thrombocytopenia and direct cytotoxicity to osteoblasts [50]. Fisetin has not been associated with any adverse effects [51]. As senolytics, these drugs would be
administered in an intermittent fashion, for example once every few weeks. This intermittent dosing strategy, when combined with the short elimination half-lives of senolytic drugs, may limit drug toxicity and those off-target effects that arise through continuous drug occupancy of a receptor or inhibition of an enzyme [46, 47]. In fact, an intermittent dosing schedule was shown to improve tolerability of dasatinib in patients with chronic myeloid leukemia [52]. Intermittent dosing may also be beneficial for medication adherence, given that senolytics can be taken for only a fraction of days out of a week or month. Theoretical adverse effects due to the clearance of senescent cells, rather than due to off targets of the drugs themselves, are at this time theoretical. However, based on preclinical data these might include negative effects on wound healing or fibrosis during liver regeneration [43, 53].

Initial clinical trials of senolytics in humans are underway, and the results of several small studies have already been published [17, 54, 55]. In an open-label pilot study of patients with idiopathic pulmonary fibrosis (IPF), senolytic (dasatinib + quercetin) therapy improved physical function, including 6 minute walk distance, gait speed, chair stand time, and the short physical performance battery [55]. In another open-label phase 2 study of patients with diabetic kidney disease, senolytic therapy reduced senescent cell burden, macrophages, and crown like structures in adipose tissue as well as blood SASP factors [54]. Additional studies are beginning or are ongoing for osteoarthritis, osteoporosis, Alzheimer’s disease, Covid-19 in hospitalized and nursing home patients, frailty in elderly women, and childhood cancer survivors, among others (ClinicalTrials.gov Identifiers: NCT 04210986, 0431363, 04685590, 04476953, 04476953, 03430037, and 04733534, respectively).
Targeting Senescent Cells Improves Diabetic Phenotypes and Complications

In diet-induced obese (DIO) mice, senescent cell clearance has many beneficial effects on adipose tissue function and systemic metabolism. After senescent cell clearance, mean adipocyte size is smaller, and adipose tissue distribution favors subcutaneous depots rather than visceral ones, without reduction of body weight [18]. Ectopic lipid deposition in muscle and liver is also decreased [18, 30]. These features are correlated with insulin sensitivity, which is improved after senescent cell clearance[18]. Senescent cell clearance in aged mice mitigates age-related fat tissue loss due to improved adipogenesis [27]. In addition to these metabolic benefits, some complications of diabetes were also improved or prevented in obese mice, including microalbuminuria, diastolic cardiac dysfunction, hepatic steatosis, and obesity-induced anxiety [18, 27, 56, 57]. Osteoarthritis, atherosclerosis, and vascular reactivity have also been shown to improve after senolytic treatment in experimental animals [58, 59]. As previously mentioned, reduction of senescent cells in adipose tissue has been seen in a small human study of senolytics, providing an indication that senolytic treatment may alleviate diabetic phenotypes in humans [54].

Little is known about the effect of current diabetes therapies on senescent cell quantity or function in humans. Certain existing therapies, including metformin and acarbose, have been shown to extend lifespan in nondiabetic mice, to a greater extent in males than females, although senescent cell burden was not measured in these studies [60]. In cell culture experiments, metformin can alleviate the SASP via activation of Nrf2-Gpx7 and NF-kB inhibition [61, 62]. Metformin has also been shown to improve proliferative capacity of cells in culture, including mesenchymal stem cells isolated from mice with chronic kidney disease [63]. In addition, metformin is known to alleviate diabetic complications and metabolic syndrome, and may confer lower cancer risk in diabetic patients [64]. However,
more studies are needed to explore whether these effects are mediated by prevention of senescence, inhibition of the SASP, or some combination of these mechanisms. Additionally, more investigation is needed to determine the impact that other diabetes therapies (i.e. SGLT-2 inhibitors, sulfonylureas, GLP-1 agonists, DPP-4 inhibitors) may have on cellular senescence and its downstream effects.

**Senolytic Clinical Trial Design**

Senolytic drugs show a great deal of promise in preclinical studies for obesity-induced metabolic dysfunction and diabetes. Careful clinical trial design is needed to test if these benefits also occur in humans and if senolytics are safe for use in humans. Studies to determine whether senolytic drugs can prevent the onset of diabetes are not feasible as a first step given the length of time needed to test that hypothesis. Optimally, initial trials should focus on conditions for which current therapies are ineffective, given that the risk/benefit profile of senolytic drugs in humans is not yet known. In the setting of diabetes, indications that could make sense to target in early trials are diabetic complications such as retinopathy, diabetic ulcers, and diabetic kidney disease, as there are limited or no current therapies to specifically treat several such complications, and these are difficult to manage once present. Investigations of senolytic effects on diabetic complications could be completed within a reasonable timeline. As senolytic drugs remove senescent, dysfunctional cells that may exacerbate diabetic complications, they may have significant therapeutic potential for these indications.

If clinical trials for serious diabetic complications, such as non-healing diabetic skin wounds, heart failure with preserved ejection fraction, nephropathy, or retinopathy, show efficacy and senolytic drug risk profiles are favorable, then additional studies could be pursued. For example, ability of senolytic therapy to prevent, rather than treat, diabetic
complications could be explored. Direct measures of insulin sensitivity, such as glucose or insulin tolerance testing or hyperglycemic clamping, or clinical measures, such as changes in required insulin dose following senolytic treatment, could be of interest. If successful, then trials focusing on the prevention of senescence-associated conditions, such as prevention of insulin resistance in obesity or prevention of diabetic chronic kidney disease, could be pursued. Of course, judicious safety monitoring is necessary, as the long-term effects of clearing senescent cells in humans are not known. The results of randomized, controlled trials must be available before senolytics are prescribed to patients outside of clinical trials.

As previously mentioned, significant investigation is needed to determine the optimal administration schedule of senolytics (i.e. weekly, monthly, bimonthly) for each individual disease state according to the rate of re-accumulation of senescent cells in that disease. Different senolytics and combinations of senolytics may need to be investigated to achieve optimal senescent cell clearance in particular tissues or diseases. There may also be patient factors, such as pharmacokinetic differences, that affect the efficacy of different senolytics for a particular subset of individuals. Targeted delivery of senolytic drugs to specific tissues could be explored in the future as a strategy. This may be especially useful in the case of localized pathologies, such as diabetic retinopathy.

Conclusions

Senescent cell burden is increased in obesity and diabetes and likely plays a role in the development of diabetes and its complications. Novel senolytic drugs have been identified that can remove senescent cells by disarming their pro-survival pathways called SCAPs. Senolytics have been shown to alleviate multiple diseases in preclinical studies, including insulin resistance and diabetic complications in animal models, and can reduce senescent cell abundance in human adipose tissue. Further preclinical studies are needed to
define the advantages or noninferiority of senolytics when compared with current diabetes therapies, as well as to demonstrate their safety. Eventually, randomized controlled clinical trials are needed to further investigate the utility of senolytics in human obesity and diabetes. As there are currently no specific therapies to alleviate diabetic complications, these might be appropriate targets for early trials of senolytic drugs in diabetic patients. Data from well-designed clinical trials is needed prior to patients receiving senolytic drugs outside of clinical trials. Senolytics represent a potential new therapeutic class for the prevention and treatment of diabetes and its complications.
Acknowledgements

The authors are supported by NIH grants R37AG013925, P01AG062413, R01AG 072301, R01DK120292, and the Translational Geroscience Network (R33AG061456), the Alzheimer’s Association Part the Cloud Program, Robert and Arlene Kogod, the Connor Group, Robert J. and Theresa W. Ryan, and the Noaber Foundation.

Data availability

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.
References:

1. Birch, J. and J. Gil, Senescence and the SASP: many therapeutic avenues. Genes Dev, 2020. 34(23-24): p. 1565-1576.
2. Iske, J., et al., Senolytics prevent mt-DNA-induced inflammation and promote the survival of aged organs following transplantation. Nat Commun, 2020. 11(1): p. 4289.
3. Coppe, J.P., et al., The senescence-associated secretory phenotype: the dark side of tumor suppression. Annual review of pathology, 2010. 5: p. 99-118.
4. Tchkonia, T., et al., Fat tissue, aging, and cellular senescence. Aging Cell, 2010. 9: p. 667-684.
5. Minamino, T., et al., A crucial role for adipose tissue p53 in the regulation of insulin resistance. Nat Med, 2009. 15(9): p. 1082-7.
6. Chen, Y.W., et al., Ablation of XP-V gene causes adipose tissue senescence and metabolic abnormalities. Proc Natl Acad Sci U S A, 2015. 112(33): p. E4556-64.
7. Kirkland, J.L. and T. Tchkonia, Cellular Senescence: A Translational Perspective. EBioMedicine, 2017.
8. Munoz-Espin, D. and M. Serrano, Cellular senescence: from physiology to pathology. Nature reviews. Molecular cell biology, 2014. 15(7): p. 482-96.
9. Xu, M., et al., Senolytics improve physical function and increase lifespan in old age. Nature Medicine, 2018. 24(8): p. 1246-1256.
10. Palmer, A.K., et al., Cellular Senescence in Type 2 Diabetes: A Therapeutic Opportunity. Diabetes, 2015. 64(7): p. 2289-98.
11. Kuki, S., et al., Hyperglycemia accelerated endothelial progenitor cell senescence via the activation of p38 mitogen-activated protein kinase. Circulation journal : official journal of the Japanese Circulation Society, 2006. 70(8): p. 1076-81.
12. Acosta, J.C., et al., A complex secretory program orchestrated by the inflammasome controls paracrine senescence. Nature Cell Biology, 2013. 15(8): p. 978-90.
13. Wang, B., et al., Transplanting cells from old but not young donors causes physical dysfunction in older recipients. Aging Cell, 2020: p. e13106.
14. Kim, S.R., et al., Transplanted senescent renal scattered tubular-like cells induce injury in the mouse kidney. Am J Physiol Renal Physiol, 2020. 318(5): p. F1167-F1176.
15. Zhu, Y., et al., The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. Aging Cell, 2015. 14(4): p. 644-58.
16. Zhu, Y., et al., Identification of a novel senolytic agent, navitoclax, targeting the Bcl-2 family of anti-apoptotic factors. Aging Cell, 2016. 15(3): p. 428-35.
17. Kirkland, J.L. and T. Tchkonia, Senolytic drugs: from discovery to translation. J Intern Med, 2020. 288(5): p. 518-536.
18. Palmer, A.K., et al., Targeting senescent cells alleviates obesity-induced metabolic dysfunction. Aging Cell, 2019: p. e12950.
19. Gustafson, B., A. Nerstedt, and U. Smith, Reduced subcutaneous adipogenesis in human hypertrophic obesity is linked to senescent precursor cells. Nat Commun, 2019. 10(1): p. 2757.
20. Hannou, S.A., et al., Functional genomics of the CDKN2A/B locus in cardiovascular and metabolic disease: what have we learned from GWASs? Trends in endocrinology and metabolism: TEM, 2015. 26(4): p. 176-84.
21. Garg, A., Clinical review#: Lipodystrophies: genetic and acquired body fat disorders. J Clin Endocrinol Metab, 2011. 96(11): p. 3313-25.
22. Caron, M., et al., Human lipodystrophies linked to mutations in A-type lamins and to HIV protease inhibitor therapy are both associated with prelamin A accumulation, oxidative stress and premature cellular senescence. Cell Death Differ, 2007. 14(10): p. 1759-67.
23. Kung, C.P. and M.E. Murphy, The role of the p53 tumor suppressor in metabolism and diabetes. J Endocrinol, 2016. 231(2): p. R61-R75.
24. Wu, D. and C. Prives, *Relevance of the p53-MDM2 axis to aging*. Cell Death Differ, 2018. 25(1): p. 169-179.
25. Huang, Q., et al., *Role of p53 in preadipocyte differentiation*. Cell Biol Int, 2014. 38(12): p. 1384-93.
26. Zaragosi, L.E., et al., *Activin a plays a critical role in proliferation and differentiation of human adipose progenitors*. Diabetes, 2010. 59(10): p. 2513-2521.
27. Xu, M., et al., *Targeting senescent cells enhances adipogenesis and metabolic function in old age*. Elife, 2015. 4: p. e12997.
28. Mitterberger, M.C., et al., *Adipogenic differentiation is impaired in replicative senescent human subcutaneous adipose-derived stromal/progenitor cells*. J Gerontol A Biol Sci Med Sci, 2014. 69(1): p. 13-24.
29. Hammarstedt, A., et al., *Impaired Adipogenesis and Dysfunctional Adipose Tissue in Human Hypertrophic Obesity*. Physiol Rev, 2018. 98(4): p. 1911-1941.
30. Ogrodnik, M., et al., *Cellular senescence drives age-dependent hepatic steatosis*. Nature communications, 2017. 8: p. 15691.
31. Helman, A., et al., *p16(Ink4a)-induced senescence of pancreatic beta cells enhances insulin secretion*. Nature Medicine, 2016. 22(4): p. 412-20.
32. Thompson, P.J., et al., *Targeted Elimination of Senescent Beta Cells Prevents Type 1 Diabetes*. Cell Metab, 2019. 29(5): p. 1045-1060 e10.
33. Krishnamurthy, J., et al., *Ink4a/Arf expression is a biomarker of aging*. J Clin Invest, 2004. 114(9): p. 1299-307.
34. Wissler Gerdes, E.O., et al., *Discovery, Development, and Future Application of Senolytics: Theories and Predictions*. The FEBS journal, 2020.
35. Wang, E., *Senescent human fibroblasts resist programmed cell death, and failure to suppress bcl2 is involved*. Cancer Research, 1995. 55(11): p. 2284-92.
36. Montero, J.C., et al., *Inhibition of SRC family kinases and receptor tyrosine kinases by dasatinib: possible combinations in solid tumors*. Clinical cancer research : an official journal of the American Association for Cancer Research, 2011. 17(17): p. 5546-52.
37. Olave, N.C., et al., *Upstream stimulatory factor-2 mediates quercetin-induced suppression of PAI-1 gene expression in human endothelial cells*. Journal of Cellular Biochemistry, 2010. 111(3): p. 720-6.
38. Bruning, A., *Inhibition of mTOR signaling by quercetin in cancer treatment and prevention*. Anti-cancer agents in medicinal chemistry, 2013. 13(7): p. 1025-31.
39. Chang, J., et al., *Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice*. Nature Medicine, 2016. 22(1): p. 78-83.
40. Zhu, Y., et al., *New agents that target senescent cells: the flavone, fisetin, and the BCL-XL inhibitors, A1331852 and A1155463*. Aging, 2017.
41. Pignolo, R.J., et al., *Reducing Senescent Cell Burden in Aging and Disease*. Trends Mol Med, 2020. 26(7): p. 630-638.
42. Tchkonia, T., A.K. Palmer, and J.L. Kirkland, *New Horizons: Novel Approaches to Enhance Healthspan Through Targeting Cellular Senescence and Related Aging Mechanisms*. J Clin Endocrinol Metab, 2020.
43. Demaria, M., et al., *An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA*. Developmental Cell, 2014. 31(6): p. 722-33.
44. Kirkland, J.L. and T. Tchkonia, *Cellular Senescence: A Translational Perspective*. EBioMedicine, 2017. 21: p. 21-28.
45. Kirkland, J.L., et al., *The Clinical Potential of Senolytic Drugs*. Journal of the American Geriatrics Society, 2017. 65(10): p. 2297-2301.
46. Christopher, L.J., et al., *Metabolism and disposition of dasatinib after oral administration to humans*. Drug metabolism and disposition: the biological fate of chemicals, 2008. 36(7): p. 1357-64.
47. Graefe, E.U., et al., Pharmacokinetics and bioavailability of quercetin glycosides in humans. Journal of clinical pharmacology, 2001. 41(5): p. 492-9.
48. Fox, L.C., et al., The incidence and natural history of dasatinib complications in the treatment of chronic myeloid leukemia. Blood Adv, 2017. 1(13): p. 802-811.
49. Andres, S., et al., Safety Aspects of the Use of Quercetin as a Dietary Supplement. Mol Nutr Food Res, 2018. 62(1).
50. Sharma, A.K., et al., The Senolytic Drug Navitoclax (ABT-263) Causes Trabecular Bone Loss and Impaired Osteoprogenitor Function in Aged Mice. Front Cell Dev Biol, 2020. 8: p. 354.
51. Maher, P., How fisetin reduces the impact of age and disease on CNS function. Front Biosci (Schol Ed), 2015. 7: p. 58-82.
52. La Rosee, P., et al., Improved tolerability by a modified intermittent treatment schedule of dasatinib for patients with chronic myeloid leukemia resistant or intolerant to imatinib. Ann Hematol, 2013. 92(10): p. 1345-50.
53. Krizhanovsky, V., et al., Senescence of activated stellate cells limits liver fibrosis. Cell, 2008. 134: p. 657-667.
54. Hickson, L.J., et al., Senolytics decrease senescent cells in humans: Preliminary report from a clinical trial of Dasatinib plus Quercetin in individuals with diabetic kidney disease. EBioMedicine, 2019. 47: p. 446-456.
55. Justice, J.N., et al., Senolytics in idiopathic pulmonary fibrosis: Results from a first-in-human, open-label, pilot study. EBioMedicine, 2019.
56. Kim, S.R., et al., Increased renal cellular senescence in murine high-fat diet: effect of the senolytic drug quercetin. Translational research : the journal of laboratory and clinical medicine, 2019.
57. Ogrodnik, M., et al., Obesity-Induced Cellular Senescence Drives Anxiety and Impairs Neurogenesis. Cell Metabolism, 2019.
58. Farr, J.N., et al., Targeting cellular senescence prevents age-related bone loss in mice. Nature Medicine, 2017.
59. Roos, C.M., et al., Chronic senolytic treatment alleviates established vasomotor dysfunction in aged or atherosclerotic mice. Aging Cell, 2016: p. Feb 10. doi: 10.1111/acel.12458. [Epub ahead of print].
60. Harrison, D.E., et al., Acarbose, 17-alpha-estradiol, and nordihydroguaiaretic acid extend mouse lifespan preferentially in males. Aging Cell, 2014. 13(2): p. 273-82.
61. Fang, J., et al., Metformin alleviates human cellular aging by upregulating the endoplasmic reticulum glutathione peroxidase 7. Aging Cell, 2018. 17(4): p. e12765.
62. Moiseeva, O., et al., Metformin inhibits the senescence-associated secretory phenotype by interfering with IKK/NF-kappaB activation. Aging Cell, 2013. 12(3): p. 489-98.
63. Kim, H., et al., Metformin inhibits chronic kidney disease-induced DNA damage and senescence of mesenchymal stem cells. Aging Cell, 2021. 20(2): p. e13317.
64. Noto, H., et al., Cancer risk in diabetic patients treated with metformin: a systematic review and meta-analysis. PLoS One, 2012. 7(3): p. e33411.