Review

MOLECULAR METABOLISM

Targeting cellular senescence in metabolic disease

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ABSTRACT

Cellular senescence is a cell fate involving cell cycle arrest, resistance against apoptosis, and the development of a secretome that can be pro-inflammatory. In aging and obesity, senescent cells accumulate in many tissues, including adipose tissue, brain, kidney, pancreas, and liver. These senescent cells and their downstream effects appear to perpetuate inflammation and have been implicated in the pathogenesis of metabolic dysfunction. Senescent cells are cleared in part by the immune system, a process that is diminished in obesity and aging, likely due in part to senescence of immune cells themselves. Targeting senescent cells or their products improves metabolic function in both aging and in animal models of obesity. Novel therapeutics to target senescent cells are on the horizon and are currently being investigated in clinical trials in humans for multiple diseases. Early evidence suggests that senolytic drugs, which transiently disarm the anti-apoptotic defenses of pro-inflammatory senescent cells, are effective in causing depletion of senescent cells in humans. Senescence-targeting therapeutics, including senolytic drugs and strategies to increase immune clearance of senescent cells, hold significant promise for treating metabolic dysfunction in multiple tissues and disease states.

Keywords Cellular senescence; Adipose; Aging; Obesity; Senolytics

1. INTRODUCTION: CELLULAR SENESCENCE IS A HALLMARK OF AGING AND DISRUPTED METABOLISM

Cellular senescence is an essentially irreversible cell fate that occurs in response to various cellular stressors and involves cell cycle arrest, chromatin rearrangement, morphologic changes, metabolic shift toward glycolysis, and release of factors comprising the senescence-associated secretory phenotype (SASP), which can include pro-inflammatory and matrix remodeling proteins [1,2]. Cellular senescence is a fundamental mechanism of aging [3]. Senescent cells accumulate in many different tissues with aging and have been implicated in a multitude of age-related diseases [1]. Triggers for senescent cell formation include DNA damage, telomere shortening, oncogene activation, metabolic signals (including high insulin, glucose, saturated fatty acids, ceramides, and ROS), mechanical stress, mitochondrial dysfunction, and infectious agents, among others [4–8].

Despite the relatively low percentage of cells that is senescent in any particular tissue, senescent cells can have far-reaching effects, likely through the action of their SASP. For example, transplanting relatively few senescent cells into adult mice causes frailty and all-cause mortality [9]. However, fewer senescent cells are needed to induce frailty in older than younger mice, as well as obese than lean mice [9]. This indicates that a minimum number or percentage of senescent cells may need to be surpassed prior to causing dysfunction, and that this minimum number may vary with individual characteristics such as age. This concept is termed the Threshold Theory of Senescent Cell Burden [10].

A large subset of senescent cells produces a proinflammatory, pro-apoptotic SASP. Composition of the SASP varies according to cell type, inducers of senescence, length of time since induction of senescence, and microenvironmental factors. The SASP may include cytokines such as IL-1α, IL-6, IL-8, TNFα, interferon-γ, chemokines, matrix remodeling factors, hemostatic factors, bioactive lipids, growth factors, as well as microRNAs, noncoding RNAs, ROS, and exosomes. The SASP may serve to spread senescence to neighboring and distant cells, or to amplify the SASP of already-senescent cells [9,11]. In addition, cell-extrinsic factors such as PAMPs and DAMPs, circulating factors, or pharmacologic agents, may cause amplification of a cell's SASP [12]. For example, the pro-inflammatory SASP can be accentuated by lipopolysaccharide (a product of gram negative bacteria) or coronavirus [6,13].

Senescent cells also attract, anchor, and activate immune cells in tissue through the SASP [7,14]. Immune cells including, but not limited to T cells, NK cells, and macrophages clear senescent cells under normal circumstances, including in young individuals [15]. Impaired immune cell cytotoxicity in experimental animals leads to accumulation of senescent cells [16]. Immune function is therefore one of many factors that likely impacts the rapidity of senescent cell accumulation. Other factors might include environmental exposures, genetic predisposition, tissue or cell type, and disease state and severity.

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Received July 4, 2022 • Revision received September 5, 2022 • Accepted September 12, 2022 • Available online 16 September 2022

https://doi.org/10.1016/j.molmet.2022.101601

Not all senescent cells acquire a pro-inflammatory SASP phenotype [17]. For example, senescent cell burden in human abdominal subcutaneous adipose tissue is positively correlated with pro-inflammatory cytokine gene expression, however in femoral fat, this correlation is not present [18]. Senescent cells are postulated to play positive roles in wound healing and prevention of fibrosis and cancers, and are also essential for normal fetal development and possibly parturition [19–21]. For these reasons, as well as the hugely important role of cellular senescence as an anti-tumor mechanism, therapies that target senescent cells should likely avoid the outright prevention of senescent cell formation. However, the utility of preventing formation of additional senescent cells, such as when a certain threshold of senescent cells is already present or when the senescent cells eliminated are those with a pro-apoptotic SASP, could be weighed against potential safety concerns. This topic requires further study.

In the remainder of this review, we will focus on the roles that cellular senescence plays in the development of metabolic dysfunction in multiple tissues and discuss current methods under development and in early clinical trials to target senescent cells in metabolic disease.

2. ORGAN-SPECIFIC IMPLICATIONS OF CELLULAR SENESCENCE ON METABOLIC HEALTH

2.1. Adipose tissue

Adipose tissue is one of the largest organs in most humans and is important for energy storage, metabolic signaling, and immunity. In the face of nutrient excess, adipose tissue expands through both hypertrophy, or enlargement of cell size, and hyperplasia, or cell division [22]. White adipose tissue is located in both subcutaneous and visceral depots and contains a large number of senescent cells, even in lean individuals [18], and senescent cell burden increases in obesity along with increased fat mass [14,23,24]. Possible inducers of cellular senescence in obesity include oxidative stress, hyperinsulinemia, hyperglycemia, fatty acids, and telomere shortening, among others [4,25]. In addition to adipose tissue, senescent cells are found in other organs including liver, muscle, pancreas, and the brain in obesity [26–28].

Preadipocytes, or adipose progenitor cells (also known as MSCs), are susceptible to cellular senescence. Preadipocytes isolated from obese humans exhibit increased senescence markers and limited replicative potential compared to preadipocytes isolated from lean individuals [29]. Progenitor cell senescence is thought to be the mechanism responsible for reduced adipogenic potential in hypertrophic obesity, and is characterized by increased adipose cell size [30]. Upregulation of p53 in obesity also prevents differentiation of preadipocytes [31].

In addition to intrinsic reduction in differentiation potential in senescent adipose progenitor cells, these senescent cells also affect the function of neighboring cells through their SASP. For example, Activin A secreted by senescent progenitor cells blunts the ability of neighboring cells to differentiate [32,33]. Activin A, as well as other SASP factors such as IL-6 and TNFα, can also directly impede insulin sensitivity in neighboring cells [34] and attract immune cells that further promote insulin resistance. Through these mechanisms, senescent cells are likely involved in the pathogenesis of type 2 diabetes, which in itself entails multiple processes that beget further cellular senescence.

Features of cellular senescence including upregulation of p53 and p21, SA-βgal activity, secretion of inflammatory factors, and accumulation of phosphorylated H_2AX (γ-H_2AX) foci, have been observed in differentiated adipose cells (adipocytes) in mice lacking the DNA polymerase η gene (*pol* $\eta^{-/-}$) [35], as well as in obese humans [36]. In *pol* $\eta^{-/-}$ mice, many of the senescent features develop prior to glucose intolerance or insulin resistance, suggesting that senescence induced by DNA damage may play a role in the development of adipose tissue dysfunction in obesity. In humans, prolonged exposure to hyperinsulinemia induces mature adipocytes to undergo cell cycle progression (endoreplication) mediated by cyclin D1, which in the presence of further hyperinsulinemia leads to activation of senescence pathways [36]. In obese humans, adipocyte senescence was significantly correlated with hyperinsulinemia, independently of age, and was more likely to occur in subcutaneous than omental adipocytes [36].

Removal of senescent cells from obese animals either through genetic means targeting $p21^{Cip1}$ or $p16^{Ink4a}$ expressing cells, or with senolytic drugs, improves whole body and adipose insulin sensitivity, indicating that senescent cells likely contribute to obesity-related metabolic dysfunction [14,37]. In addition, several measures of adipose tissue function improve with senescent cell removal, including decreases in adipocyte size, improved adipogenic potential, and decreased macrophage infiltration [14,33,37]. In studies involving transplantation of perigonadal adipose tissue from obese mice into lean mice, selective removal of $p21^{Cip1}$ highly expressing cells from transplanted tissue mitigated the metabolic dysfunction typically induced by the transplanted tissue. Similarly, senolytic treatment of adipose tissue from obese humans prior to transplantation into immunodeficient mice also prevented the development of insulin resistance seen in mice that received adipose tissue transplants without senolytic treatment [37]. Taken together, these studies implicate adipose tissue senescent cells in development of obesity related metabolic dysfunction.

2.2. Liver

In obesity, the liver is the main site of ectopic lipid deposition, which is associated with cellular senescence [27,38]. Senescent cell abundance correlates with severity of fibrosis, diabetes, and adverse outcomes in individuals with non-alcoholic fatty liver disease [38]. Independently of NAFLD, markers of senescence in liver biopsies were correlated with hyperinsulinemia in patients undergoing bariatric surgery [4], and prolonged exposure to hyperinsulinemia induces senescence or promotes the SASP in already senescent human hepatocytes *in vitro* [39]. Therefore, hyperinsulinemia in obesity is a driver of hepatic senescence.

Senescent cells have also been implicated as a key player in the gut-liver axis in obesity-related hepatocellular carcinoma (HCC), which is associated with cellular senescence of hepatic stellate cells (HSCs). The gut microbiome in obesity promotes HSC senescence through increased secretion of deoxycholic acid, a metabolite that can cause DNA damage, and lipoteichoic acid, which seems to enhance the SASP of HSCs [40]. Reduction of gut bacteria or inhibition of DCA production, as well as reduction of senescent HSCs, reduced HCC formation in obese mice [41]. More study is needed to determine whether senescent cells are a viable target for reducing HCC risk in human obesity.

2.3. Pancreas

Features of cellular senescence including $p16^{INK4a}$ expression, reduced proliferative capacity, β-galactosidase activity, SASP production, and upregulation of SCAPs are found in pancreatic beta cells (β -cells) with aging and obesity [42–44]. Although $p16^{Ink4a}$ expression is an established cause of reduced β-cell proliferation with aging, its role in insulin secretion is less clear. For example, β-cell insulin secretion increased after overexpression of $p16^{Ink4a}$ in young mice [42], however a separate study showed improved β-cell function and insulin secretion following removal of $p16^{Ink4a}$ highly expressing cells [43]. In a mouse model of type 1 diabetes, senolytic treatment reduced the immune destruction of β-cells, possibly due to reduction in the SASP, which resulted in improved insulin secretion [45]. The role of

cellular senescence may vary in different forms of diabetes, and more work is needed to define the role of cellular senescence in pancreatic beta cell function.

3. OPPORTUNITIES AND METHODS TO TARGET SENESCENT CELLS

3.1. Geroscience Hypothesis

Cellular senescence is one of many recognized hallmarks of aging thought to be linked to multiple or perhaps all chronic diseases of aging [3,46]. The "Geroscience Hypothesis" posits that by targeting fundamental aging mechanisms, we may delay, prevent, alleviate, or treat many chronic disorders and diseases at once. This could lead to a larger impact on extending the period of life without serious disease or disability ("healthspan") than targeting one specific disease at a time. Cellular senescence is an attractive therapeutic target to test the geroscience hypothesis, given that it has been implicated in the pathogenesis of diverse diseases. Efforts are also underway to utilize metformin, which, among other effects, inhibits the SASP of senescent cells [47], to target aging broadly (see below and [48]). Several strategies are currently under study to target senescent cells including senolytics, SASP inhibitors, and enhancers of immune clearance.

3.2. Senolytic drugs

Recently, pharmacologic therapies to target senescent cells were discovered through the knowledge that deleterious senescent cells can upregulate anti-apoptotic pathways called senescence-associated antiapoptotic pathways, or SCAPs [49,50]. By inhibiting SCAPs, these agents, termed senolytics, allow senescent cells to undergo apoptosis in response to their own pro-apoptotic *milieu*, leading to clearance of those senescent cells that have a pro-inflammatory SASP from tissues. These drugs work much like antibiotics or chemotherapeutics by clearing a subset of cells provided they achieve therapeutic levels, and therefore do not require prolonged, continual administration. The intermittent, "hit and run" administration schedule of senolytics leads to lower risk of side or off-target effects and may also lead to improved medication adherence.

Senolytic drugs impact other aging mechanisms including restoration of NAD^+ levels through reversing age-related declines in CD38 [51], reducing age related inflammation and fibrosis [52–54], decreasing DNA damage, and restoring progenitor cell function in multiple tissues [33,55,56]. This is consistent with the Unitary Theory of Targeting Fundamental Aging Mechanisms, which postulates that most or all fundamental aging mechanisms would be positively impacted by targeting any one fundamental aging mechanism [10].

Preclinical studies of senolytic drugs in experimental animals have shown positive impact on obesity-related adipose tissue dysfunction, insulin resistance, anxiety, and kidney dysfunction, frailty, cardiac fibrosis, liver steatosis, survival after coronavirus infection, osteoporosis, pulmonary fibrosis, intervertebral disc degeneration, and healthspan [9,13,14,26,27,53,55,57–61]. *In vitro*, senolytic drugs inhibit hyperinsulinemia-induced senescence in human hepatocytes [39] and clear senescent cells from human adipose tissue explants [9].

Findings in early open-label clinical trials indicate that systemic administration of senolytic drugs can clear senescent cells in human tissue. For example, a 3-day treatment course with dasatinib and quercetin reduced $p16^{INK4a}$ and $p21^{CIP1}$ expressing cells in adipose tissue and epidermis in humans with idiopathic pulmonary fibrosis (IPF) [62]. In another open label pilot study in individuals with IPF, senolytic treatment improved several measures of physical function including 6-minute walk distance, gait speed, chair stand time, and short physical performance battery [63]. Multiple studies are underway to explore the effects of senolytics in other tissues and age-related conditions (*e.g.*, NCT03675724, NCT05276895, NCT04063124, NCT04685590, NCT04733534, NCT04903132, NCT04313634). Randomized controlled trials and additional safety data are needed to further elucidate the risks and benefits of senolytic therapies.

3.3. SASP inhibitors ("senomorphic" drugs)

Rather than eliminate senescent cells directly, several drugs have been investigated that mitigate the effects of the SASP, in turn reducing the spread of senescence from one cell to another, or allowing clearance of senescent cells by immune cells due to removal of inhibitory signals. Drugs that belong to this category include metformin, rapamycin, ruxolitinib, and p28MAPK inhibitors [33,34,47,64–66]. In general, these drugs reduce some, but not all, SASP factors. Some drugs seem to have senolytic effects in certain cell types and senomorphic effects in others [67].

3.4. Enhancing immune clearance

Multiple changes with aging affect the ability of immune cells to effectively clear senescent cells. This includes changes to the microenvironment in organs where immune cells differentiate, mature, or circulate, such as bone marrow, thymus, lymph nodes, and spleen [68]. Chronic, sterile inflammation (*i.e.*, not associated with infection) increases with aging, with contributions from circulating components of the SASP, and likely inhibits immune cell function [69]. Therefore, blocking the SASP is one strategy to allow immune cells to clear senescent cells. In addition, other more targeted strategies to enhance immune cell recognition and destruction of senescent cells are under investigation. For example, vaccination in mice against GPNMB, a transmembrane protein involved in endothelial cell adhesion, resulted in reduced senescent cell burden and improved glucose tolerance in mice [70].

3.5. Nonpharmacologic interventions

Lifestyle modifications including healthy diet and exercise are often recommended as promoting healthy aging, and early evidence indicates that these interventions may impact senescent cell burden. In a mouse model of diet-induced obesity, exercise prevented accumulation of senescent cells and their SASP in visceral adipose tissue and improved physical function [23]. Exercise also reduced liver senescent cells in a mouse model of spontaneous liver inflammation and steatosis [71]. In humans, aerobic fitness (as measured by age-adjusted VO_{2max}) and increased physical activity are associated with lower abundance of senescent peripheral blood T-cells [72,73]. A 12-week strength and endurance exercise intervention in older adults resulted in lower circulating senescence-related proteins as well as T-cell markers of senescence including p16, p21, cGAS, and TNFα, in addition to improved physical functioning and patient-reported quality of life [74]. Older mice have much less skeletal muscle plasticity in response to exercise than younger animals, are refractory to resistance exercise alone. Importantly, combining senolytics with exercise decreased acute senescent cell accumulation due to resistance exercise and facilitated skeletal muscle hypertrophy in older animals, making their exercise response more like that of younger mice [75]. Future clinical trials of combining senolytics with exercise in older humans could yield valuable insights.

Although high-fat feeding in experimental models has been established to promote accumulation of senescent cells [14,23], a more nuanced understanding of the contributions of different dietary compositions on cellular senescence requires further study. Caloric restriction (CR), long known to extend lifespan in species ranging from flies to primates, has

wide-ranging impacts on fundamental aging mechanisms including cellular senescence, albeit accompanied by undesirable side effects including hunger, reduced body temperature, and reduced libido [76,77]. CR reduces senescence markers in multiple tissues in mice and in colon of healthy humans [78,79], likely through multiple mechanisms including reduction of senescence inducers such as oxidative stress, downregulation of SASP factors, and upregulation of autophagy [77]. More study is needed to determine whether other approaches to dietary restriction such as intermittent fasting, which has been more widely adopted than CR for practical reasons, have effects on cellular senescence. Additionally, as is the case with exercise, it will be important to determine whether dietary interventions have additive or synergistic effects on senescence when combined with senolytic or senomorphic therapies.

3.6. Challenges for clinical trials targeting senescent cells

Standard biomarkers for aging have not been established, although expert panels have convened to tackle a framework for clinical trials going forward [80,81]. In particular, detection of senescent cells is challenging due to lack of universal markers of cellular senescence, owing to lack of adequate sensitivity and specificity of individual markers, such as $p16^{INK4a}$ expression or senescence-associated beta galactosidase (SA-βgal) activity. More work is needed to identify reliable markers of senescence that are shared among cell types and not present in non-senescent cells. It is also important to recognize the heterogeneity of senescence markers among tissue and cell types which may allow for improved disease-specific targeting of senescent cells [82]. Additionally, reliable markers of senescence that can be measured noninvasively, such as in blood or urine, are needed. Recently, urine levels of α-Klotho, an endocrine transmembrane protein considered to be a suppressor of aging, were shown to correlate with senescent cell burden in the brain and kidney in mice [83]. Treatment with senolytic drugs restored α-Klotho levels in kidneys, brain, and urine in mice, and in urine of patients with idiopathic pulmonary fibrosis (IPF) [83].

4. CONCLUSIONS

Senescent cells accumulate in multiple tissues in obesity and aging including adipose tissue, liver, and pancreas, and are implicated in the development of metabolic dysfunction and disease. Despite a relatively small percentage of cells that are senescent in tissue, senescent cells can have widespread impact through the actions of their SASP, which can cause propagation of senescence to neighboring cells, promote inflammation and tissue remodeling, and recruit, activate, and anchor immune cells. Senescent cells are resistant to apoptosis due to upregulation of senescence-associated anti-apoptotic pathways (SCAPs), however inhibition of these SCAPs by senolytic drugs has been found to clear senescent cells from tissue of experimental animals, and in small open-label trials, of human subjects. In addition, other senescence-targeting, or senotherapeutic, strategies are under development including SASP inihibition and immunization against senescent cells. More investigation of nonpharmacologic strategies to reduce senescent cell burden is needed. Senotherapeutic strategies have immense potential to alleviate metabolic dysfunction in multiple tissues and in turn promote extension of healthspan.

AUTHOR CONTRIBUTIONS

AKP, TT, and JLK contributed equally to this work and are accountable for the content of this work.

FUNDING

The authors are supported by NIH grants R37AG013925, P01AG062413, R01AG072301, R01DK120292, and the Translational Geroscience Network (R33AG061456), the Alzheimer's Association Part the Cloud Program, the Connor Group, Robert J. and Theresa W. Ryan, and the Noaber Foundation.

CONFLICT OF INTEREST

AKP, TT, and JLK 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.

DATA AVAILABILITY

No data was used for the research described in the article.

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