Senolytics and Senostatics: A Two-Pronged Approach to Target Cellular Senescence for Delaying Aging and Age-Related Diseases

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Aging is the most important single risk factor for many chronic diseases such as cancer, metabolic syndrome, and neurodegenerative disorders. Targeting aging itself might, therefore, be a better strategy than targeting each chronic disease individually for enhancing human health. Although much should be achieved for completely understanding the biological basis of aging, cellular senescence is now believed to mainly contribute to organismal aging via two independent, yet not mutually exclusive mechanisms: on the one hand, senescence of stem cells leads to exhaustion of stem cells and thus decreases tissue regeneration. On the other hand, senescent cells secrete many proinflammatory cytokines, chemokines, growth factors, and proteases, collectively termed as the senescence-associated secretory phenotype (SASP), which causes chronic inflammation and tissue dysfunction. Much effort has been recently made to therapeutically target detrimental effects of cellular senescence including selectively eliminating senescent cells (senolytics) and modulating a proinflammatory senescent secretome (senostatics). Here, we discuss current progress and limitations in understanding molecular mechanisms of senolytics and senostatics and therapeutic strategies for applying them. Furthermore, we propose how these novel interventions for aging treatment could be improved, based on lessons learned from cancer treatment.

Keywords: age-associated inflammation, aging, cellular senescence, senescence-associated secretory phenotype, senolytics, senostatics

INTRODUCTION

Aging is defined as a progressive decline of physiological tissue function and closely associated with many chronic human diseases (Lopez-Otin et al., 2013). Advanced age steeply increases the risk for developing cancer, cardiovascular disease, neurodegenerative disorders, osteoporosis, osteoarthritis, and metabolic syndrome (Campisi, 2013; Childs et al., 2015; Munoz-Espin and Serrano, 2014). These chronic diseases are main causes of morbidity and mortality in human, becoming the center of attention for medical research to be treated for enhancing human health. Targeting diseases individually paid off relatively well, in some sense, as human lifespan continues to increase during the last several decades. Curing most, if not all, chronic diseases separately, however, seems to be impractical and even if possible, total cost for achieving it should be taken into consideration (Campisi et al., 2019). Thus, now is the time to find a more efficient and cost effective way to treat multiple chronic diseases. Since aging is the most important single risk factor for many chronic diseases, delaying the aging process could be such an 'all-in-one' option.

To achieve such a daunting task, a better understanding of the basic aging process is instrumental. Although still far

from being complete, significant progress has been recently made to get a foot in the door to develop novel therapeutic strategies for delaying aging (Childs et al., 2015; 2017; He and Sharpless, 2017; McHugh and Gil, 2018; Niedernhofer and Robbins, 2018; van Deursen, 2019). A classic reductionist approach identified cellular senescence (hereinafter referred to as senescence), a well-studied cellular aging process (Kuilman et al., 2010; Munoz-Espin and Serrano, 2014; Salama et al., 2014), to be increased during the progression of several chronic diseases and aging itself. This led to an intriguing hypothesis that senescence contributes to aging, and thus the elimination of senescent cells might delay the aging process. In fact, a seminal study from the van Deursen group provided the first genetic evidence, supporting a causative role of senescence in modulating aging and age-related pathologies (Baker et al., 2011; 2016). They elegantly generated the INK-ATTAC mouse model carrying a drug-inducible caspase driven by the p16^{INK4A} promoter. Upon the administration of a synthetic drug called rapalog, p16^{INK4A} positive senescent cells are removed in this system by apoptosis. The effects of the elimination of senescent cells were simply striking. Not only does it improve several age-related pathologies including lipodystrophy, glomerulosclerosis, heart hypertrophy, and cataracts, but it also extends the lifespan of both progeroid BubR1 mice and naturally aged mice (Baker et al., 2011; 2016). These studies opened an entirely new area of aging research that includes new therapeutic strategies for healthspan: targeting the detrimental effects of senescence. In this review, we present a framework encompassing the molecular mechanism by which senescence can be targeted with a focus on how to improve these novel interventions for aging treatment with lessons learned from cancer treatment.

CELLULAR SENESCENCE: A STRESS-ACTIVATED CELLULAR AGING PROGRAM WITH SYSTEMIC EFFECTS THROUGH ITS SECRETORY PHENOTYPE

Many aging-related stresses including telomere shortening and oxidative stress can activate senescence, an irreversible cell cycle arrest mainly modulated by the p53/p21 and p16/Rb pathways (Campisi, 2013; He and Sharpless, 2017; Kuilman et al., 2010; Munoz-Espin and Serrano, 2014). In addition, senescent cells display several other phenotypic alterations including altered metabolism, senescent hypertrophy, and extensive changes in gene expression characterized by a massive secretory phenotype called the senescence-associated secretory phenotype (SASP) or senescence messaging secretome (SMS) (Acosta et al., 2008; Coppe et al., 2008; Kuilman et al., 2008). Senescence acts a strong tumor suppressive mechanism due to its growth arrest phenotype, yet recent studies indicated that senescence can affect a variety of biological processes including paracrine senescence, tissue repair, immune surveillance, chronic inflammation, and organismal aging. Currently, such pleiotropic effects of senescent cells are believed to be mainly attributed to their secretory phenotype, which has a huge impact on their neighboring cells, tissue microenvironment, and systemic regulation (He and Sharpless, 2017; Lopez-Otin et al., 2013; Munoz-Espin and Serrano, 2014).

How does senescence cause aging and age-related pathologies? There are at least two possibilities, although they are not mutually exclusive. The first one is senescence of tissue stem cells, which leads to stem cell exhaustion, the subsequent decline of stem cells' ability for tissue renewal, and eventually tissue deterioration and aging. Another is that if senescent cells become accumulated, at least in part, due to either decreased immune function or continuous stress stimuli, it causes chronic inflammation through the uncontrolled activation of the SASP, which leads to tissue dysfunction and eventually aging and age-related pathologies (Gorgoulis et al., 2019; He and Sharpless, 2017; Hernandez-Segura et al., 2018). Currently, both seem to work *in vivo*. For example, a reduction of hematopoietic stem cells (HSCs) repopulating capacity is well correlated with an increase of senescent HSCs. Knockout of p16^{INK4A}, one of the key regulator of senescence, partially rescued these stem cell exhaustion and functional decline (Janzen et al., 2006). This stem cell exhaustion-driven aging phenotype is widespread for many tissues including neural stem cells, mesenchymal stem cells, and satellite cells (McHugh and Gil, 2018).

In vivo evidence to support the second possibility mostly depends on correlative studies until recently. For example, it has been shown that there are increased levels of several SASP factors such as IL-1A and IL6 in tissues during aging. Inhibition of a key regulator of the inflammatory response (Doles et al., 2012; Pietras et al., 2016), NF-κB, delays DNA damage-induced senescence and aging in mice (Tilstra et al., 2012). The van Deursen group's studies with the INK-ATTAC also hints how critically the SASP contributes to the aging process (Baker et al., 2011; 2016). In their system, if stem cells become a target of senescence, they will be removed by apoptosis instead of senescence and thus stem cell exhaustion still occurs. Despite this, the selective elimination of senescent cells still mitigates several age-associated pathologies and extends lifespan in mice, emphasizing that persistence of senescent cells play a major role in the aging process. It is plausible to assume that senescent cells exert cell-non autonomous effects on neighboring cells and microenvironment, which deteriorates tissue function and eventually leads to aging. A prime suspect is the SASP, which contains a variety of intercellular signaling proteins such as proinflammatory cytokines, chemokines, growth factors, and proteases (Coppe et al., 2008). It still remains to be proved whether the SASP directly drives the aging process or not and if so, how it works. As we recently gained new insights into SASP regulation (Chien et al., 2011; De Cecco et al., 2019; Dou et al., 2017; Freund et al., 2011; Gluck et al., 2017; Kang and Elledge, 2016; Kang et al., 2015; Kwon et al., 2017; Tasdemir et al., 2016; Yang et al., 2017), however, it is a matter of time to get solid answers for such important questions.

SENOLYTICS ARE A TARGETED ELIMINATION OF SENESCENT CELLS FOR DELAYING AGING AND AGE-RELATED DISEASES

Since senescence is firmly established to be a main causal factor for aging and genetically removing senescent cells has been shown to delay several age-associated pathologies,

it is reasonable to seek strategies to target it for enhancing health. In several respects, senescent cells are like cancer cells that do not divide, including metabolic shift, epigenetic change, and resistance to apoptosis (Campisi, 2013). Thus, an initial approach to challenge this problem was similar to one that has succeeded at least partially for cancer treatment.

The Kirkland group examined the gene expression profiling with RNA sequencing between normal and senescent cells (Zhu et al., 2015). With network-level analysis, they found that senescent cells increase several networks of anti-apoptotic regulators including dependence receptors, PI3K/Akt pathway components, and BCL-2 family members, which collectively confer resistance to apoptosis. This finding led to the identification of the first generation of senolytics, agents that inhibit a portion of these pathways and induce apoptosis preferentially in senescent cells: dasatinib and quercetin induce apoptosis of certain types of senescent cells, with the former being more effective for senescent fat cell progenitors and the latter being more effective for senescent endothelial cells. More importantly, the combination of dasatinib and quercetin alleviates several senescence-associated phenotypes in both damage-induced progeria and naturally aged mice, demonstrating the feasibility of the senolytic application for enhancing healthspan (Zhu et al., 2015).

After this initial success of dasatinib and quercetin, the same group reported that navitoclax, a BCL-2 family inhibitor, can act as another class of senolytics (Zhu et al., 2016). As in the case of dasatinib and quercetin, navitoclax seems to sensitize some but not all types of senescent cells, with being effective against human umbilical vein epithelial cells (HUVECs) and IMR90 human lung fibroblasts but not for primary preadipocytes. Navitoclax targets BCL-2, BCL-xL, and MCL-1 and its efficacy in different types of senescent cells is

well correlated with expression levels of those targets. Taken together, these studies indicate two critical considerations for developing and applying senolytics: first, it is necessary to target a whole pathway for survival of senescent cells, not a single gene in that pathway as other components in the same pathway can compensate for its loss. Second, aiming for one survival pathway alone is not sufficient to eliminate all different populations of senescent cells as they are quite heterogenous similarly to cancer cells. Therefore, future studies will need to collectively identify additional pathways that different types of senescent cells depend on for survival and develop therapeutic strategies to target those for eliminating the resistant population.

After these proof-of-concept studies, senolytics were extended to apply for several age-related disease models (van Deursen, 2019)(Fig. 1); treatment with senolytic drugs have been shown to delay or ameliorate cancer metastasis (Demaria et al., 2017), idiopathic pulmonary fibrosis (Schafer et al., 2017), atherosclerosis (Childs et al., 2016), liver cirrhosis (Krizhanovsky et al., 2008; Ogrodnik et al., 2017), glomerulosclerosis (Valentijn et al., 2018), diabetes (Thompson et al., 2019), sarcopenia (Xu et al., 2018), and osteoarthritis (Jeon et al., 2017). Newly developed senolytic drugs were employed for these studies in addition to dasatinib + quercetin and navitoclxax. Some of them shared their molecular targets with the first generation of senolytic drugs. For example, ABT-737, A1331852, and A1155463 inhibits BCL-2 family members, as in the case of navitoclax (Zhu et al., 2017). Others, however, were generated to target new survival pathways in senescent cells. For example, a recent study showed that FOXO4 drives nuclear localization of p53 to suppress its engagement with mitochondrial apoptotic pathway. Based on this, a FOXO4 inhibitor peptide (FOXO4-DRI peptide) was
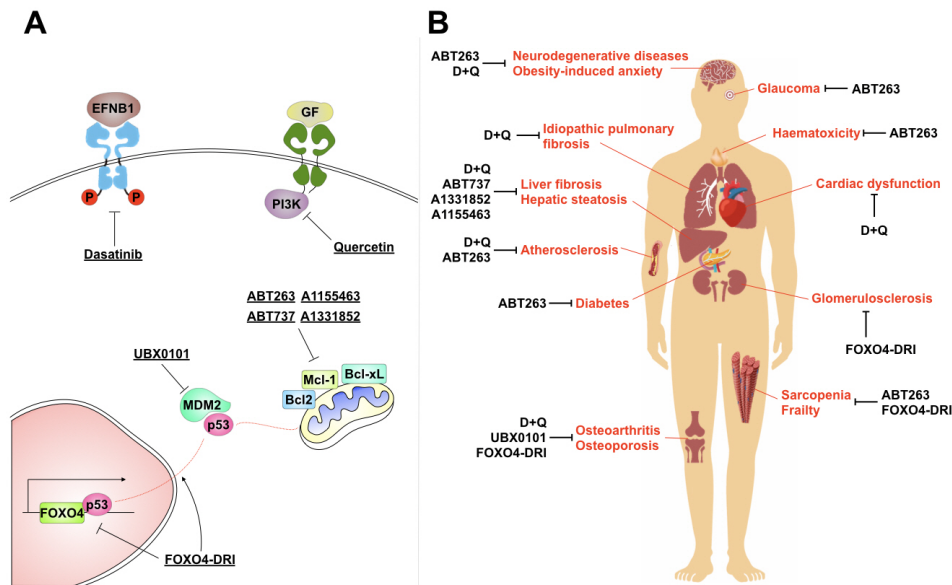
Fig. 1. Current status of senolytics and senostatics. (A) Pathways targeted by currently available senolytic and senostatic drugs. (B) Human diseases have been shown to be delayed by senotherapy (modified from freepik.com). EFNB1, ephrin B1; GF, growth factor; PI3K, phosphoinositide 3-kinases; MDM2, mouse double minute 2 homolog; Bcl2, B-cell lymphoma 2; Mcl-1, myeloid cell leukemia 1; Bcl-xL, B-cell lymphoma-extra large; FOXO4, forkhead Box O4; D + Q, co-treatment of dasatinib and quercetin.

designed to sensitize senescent cells and delay several age-associated phenotypes in mice including frailty, renal dysfunction, and hair growth (Baar et al., 2017). It will be interesting to see whether combinatorial treatment of FOXO4-DRI peptide and navitoclax or dasatinib + quercetin has a synergistic effect on the clearance of senescent cells.

Although this series of promising results, the current version of senolytics has its own limitation. Since most senolytic drugs were repositioned to target aging and age-related diseases, they might have undesirable side effects for long-term use. Navitoclax is already shown to cause severe thrombocytopenic and neutropenic effects, preventing it from being used for targeting aging (McHugh and Gil, 2018; Niedernhofer and Robbins, 2018). Therefore, it is required to reevaluate the safety of a senolytic drug for long-term use if it is originally designed for short-term use. Another potential problem is tissue atrophy, resulting from a massive removal of senescent cells by senolytic drugs. Accumulation of senescent cells varies between tissues during normal aging, being 2% to 5% on average for most tissues, yet up to 25% in the fat tissue. Senescent cells play a role at least in part to support the structure of old tissues and thus their abrupt removal may lead to atrophy, depending on the levels of senescent cells that reside in the tissue (He and Sharpless, 2017). In addition, senolytics wipes out not only deleterious effects of senescent cells but also their beneficial effects; cellular senescence contributes to wound healing (Demaria et al., 2014), cellular reprogramming (Mosteiro et al., 2016), and tissue regeneration (Ritschka et al., 2017), suggesting that complete elimination of senescent cells may hamper these beneficial effects of senescent cells. In addition, not all senescent cells lose their biological functions, indicating that keeping them may be necessary under certain conditions. For example, senescence of pancreatic β-cells enhances their insulin secretion, compensating their decreased proliferation during aging (Helman et al., 2016). Therefore, careful consideration must be given to apply senolytics and it is necessary to improve the current version of senolytics. Achieving these could be two-fold; 1) targeting another critical aspect of senescent cells, the SASP, to selectively suppress the deleterious effects of senescence (senostatics) and 2) increasing the specificity of senolytics in a designed manner.

SENOSTATICS ARE MODULATING A PROINFLAMMATORY SENESCENCE SECRETOME, SUPPLEMENTING SENOLYTICS FOR TARGETING AGING AND AGE-RELATED DISEASES

Two different strategies can be considered for targeting the SASP: modulating the SASP regulatory network in senescent cells (generalized senostatics) and inhibiting a specific component of the SASP that exerts deleterious effects (precision senostatics). For generalized senostatics, several therapuetic targets have been recently emerged as the SASP regulatory network was genetically dissected: NF-κB (Chien et al., 2011), p38 (Freund et al., 2011), GATA4 (Kang et al., 2015), mTOR (Herranz et al., 2015; Laberge et al., 2015), BRD4 (Tasdemir et al., 2016), and cGAS/STING (Dou et al., 2017; Gluck et al., 2017; Yang et al., 2017). One potential problem for target-

ing such factors is that they have also non-senescence related functions; NF-κB plays an essential role in controlling acute inflammatory response and immune response (Chien et al., 2011); p38 is involved in several stress responses including DNA damage, heat shock, and osmotic shock responses (Freund et al., 2011); GATA4 plays a major role in controlling embryonic development (Molkentin et al., 1997); mTOR governs cell growth, proliferation, protein synthesis, and autophagy (Herranz et al., 2015; Laberge et al., 2015); BRD4 functions as an epigenetic reader to modulate lineage- and cellular state-specific transcription (Tasdemir et al., 2016); finally, cGAS-STING is a component of the innate immune system that senses the presence of cytosolic DNA (Yang et al., 2017). Therefore, care must be considered to tailor therapeutic strategies to exclusively blunt the SASP regulation but no other functions. For example, GATA4 is modulated by selective autophagy in a senescence specific manner. Elucidating how selective autophagy regulates GATA4 will be helpful to design for specifically targeting the GATA4-SASP pathway without disruption of its developmental roles (Kang and Elledge, 2016; Kwon et al., 2017; Mazzucco et al., 2017).

Precision senostatics have not been seriously considered yet mainly because the composition of the SASP highly varies, depending on the cell type, the stage of senescence (early versus late senescence), and the type of senescence-inducing stimuli (De Cecco et al., 2019; Hernandez-Segura et al., 2017; Hoare et al., 2016; Wiley et al., 2016). Moreover, the composition of the SASP is quite complex, having up to a slightly less than hundred factors. Future studies need to categorize SASP factors according to their functions in a context-dependent manner. This seems to be a daunting task, yet will be very fruitful when accomplished as it makes possible to suppress deleterious effects of the SASP while keeping its beneficial roles. In fact, recent studies begin to reveal a component specific role of the SASP in several biological processes including wound healing (Demaria et al., 2014), paracrine senescence (Acosta et al., 2013), and cellular reprograming (Mosteiro et al., 2016).

LESSONS FROM CANCER TREATMENT, WHICH CAN BE APPLIED TO IMPROVE SENOLYTICS AND SENOSTATICS

As senescent cells and cancer cells are similar to each other in several aspects and much effort has been already made to treat cancer, it is plausible to apply lessons we learned from cancer treatment to improve aging (or, more precisely, senescence) treatment (Fig. 2): non-senescence addiction (Luo et al., 2009b), combinatorial drug treatment (Al-Lazikani et al., 2012), and senescence immunotherapy (Sharma and Allison, 2015).

1) Non-senescence addiction (Fig. 2A): the concept of non-oncogene addition is based on the fact that the tumorigenic state depends on the activities of several stress response pathways, which are not inherently oncogenic themselves. Targeting such pathways make cancer cells vulnerable with a minimal harmful effect on normal cells. Inhibiting the ubiquitin proteasome system (UPS) is one of the best examples for utilizing non-oncogene addiction as cancer cells normally
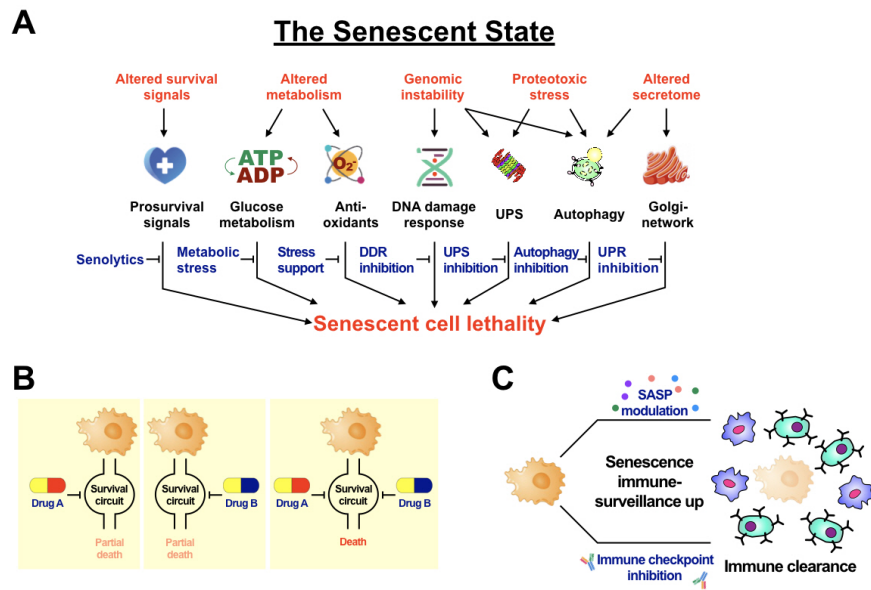
Fig. 2. Lessons from cancer treatment that help to improve senolytics and senostatics. Three new strategies to improve senotherapy adapted from the concepts that previously succeeded for cancer treatment: 1) non-senescence addiction (A), 2) combinatorial drug treatment (B), and 3) senescence immunotherapy (C).

display aneupoloidy and copy number variation, experiencing higher levels of proteotoxic stress than normal cells (Luo et al., 2009b). Senescence gene expression profiling reveals that senescent cells seem to heavily depend on pathways including Golgi vesicle transport, autophagy, and the UPS (Hernandez-Segura et al., 2017). In fact, recent studies showed that inhibiting autophagy sensitizes therapy-induced senescent cells by activating endoplasmic-reticulum-related apoptosis (Dorr et al., 2013). It will be interesting to target other stress support pathways of senescent cells to utilize non-senescence addiction for senotherapy. To expand this concept, it is possible to seek for synthetic lethal genetic interaction in senescent cells as in the case of cancer cells (Luo et al., 2009a). One potential hurdle for such genetic screens is that senescent cells do not proliferate, making it hard to maintain adequate representation of the library at each step of pooled screens. Employing a focused genetic library that targets pathways differentially expressed in senescent cells might take a detour for that problem.

2) Combinatorial drug treatment (Fig. 2B): single-agent treatment for cancer often leads to drug resistance and tumor recurrence, decreasing its efficacy for treating cancer. This mainly results from either selecting resistant population already existed or activating additional pathways to survive upon drug treatment. Combinatorial drug treatment has been proved to reduce drug resistance and also decrease potential side effects by achieving equal or better efficacy at lower doses for cancer therapies (Al-Lazikani et al., 2012). Since senescent cells also display resistance for most of senolytic drugs currently available, it is reasonable to apply combinatorial drug treatment for senescence. Two different approaches are possible here: candidate approach and unbiased drug screen. For the candidate approach, it is necessary to analyze the resistant population with gene-expression pro-

filing to identify which pathway is activated to protect them from a particular senolytic drug. Targeting such a pathway together with a senolytic drug originally applied will decrease resistance and increase drug efficacy. For the unbiased drug screen, a drug displaying the synergism with a particular senolytic drug can be identified from a traditional drug screen.

3) Senescence immunotherapy (Fig. 2C): cancer immunotherapy boosts our immune system up to target cancer by modulating immune checkpoints (Sharma and Allison, 2015). Since senescent cells can be recognized and eliminated by the immune system including macrophage, natural killer cells, and T cells, immunotherapy may also work for senolytics (McHugh and Gil, 2018). Two different strategies can be applied for senescence immunotherapy: 1) increasing the immune surveillance of senescent cells by artificially activating the SASP, which recruits immune cells for removing senescent cells (He and Sharpless, 2017) and 2) inhibiting the immune checkpoint that suppresses recognition and elimination of senescent cells by immune cells. The later could be achieved by inhibiting expression of either immunomodulatory membrane proteins or immune-suppressive components of the SASP. Insights from studies dissecting the interaction between immune cells and cancer cells will be helpful for characterizing such candidate factors in senescence immunotherapy.

CONCLUSION

Recent rapid progress in senolytics revolutionizes the field of aging research and makes it realistic to develop therapeutic strategies for delaying human aging (van Deursen, 2019). Although our current understanding of senotherapy is not complete yet, it is a matter of time to improve it through the development of new senolytic and senostatic approaches.

With more weapons in our hands, one day it might be possible to treat many age-related diseases as a manner of 'one-size-fits-all' and eventually enhance healthy human lifespan.

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