AGING

Senolytic therapies for healthy longevity

Clearing senescent cells with targeted drugs could combat age-associated disease

By **Jan M. van Deursen**

The estimated "natural" life span of humans is ~30 years, but improvements in working conditions, housing, sanitation, and medicine have extended this to ~80 years in most developed countries. However, much of the population now experiences aging-associated tissue deterioration. Healthy aging is limited by a lack of natural selection, which favors genetic programs that confer fitness early in life to maximize reproductive output. There is no selection for whether these alterations have detrimental effects later in life. One such program is cellular senescence, whereby cells become unable to divide. Cellular senescence enhances reproductive success by blocking cancer cell proliferation, but it decreases the health of the old by littering tissues with dysfunctional senescent cells (SNCs). In mice, the selective elimination of SNCs (senolysis) extends median life span and prevents or attenuates age-associated diseases (*1*, *2*). This has inspired the development of targeted senolytic drugs to eliminate the SNCs that drive age-associated disease in humans.

Much of our current knowledge about the properties of SNCs is based on experiments in cultured cells, largely because SNCs in tissues and organs are difficult to identify and collect. One key characteristic of SNCs is that they are in a state of permanent cell-cycle arrest, typically initiated and maintained by the p53-p21-retinoblastoma (RB) and p16-RB tumor suppressor pathways (*3*). Various stresses induce this state, including oxidative and genotoxic stress, shortening of telomeres (repetitive sequences that protect the ends of chromosomes), excessive mitogenic signaling, DNA replication errors, mitotic defects, and mitochondrial dysfunction. Furthermore, SNCs produce a bioactive "secretome," referred to as the senescence-associated secretory phenotype (SASP) (*4*). This can disrupt normal tissue architecture and function through diverse mechanisms, including recruitment of inflammatory immune cells, remodeling of the extracellular matrix, induction of fibrosis, and inhibition of stem cell function (*3*). Paradoxically, although cellular senescence has evolved as a tumor protective program, the SASP can include factors that stimulate neoplastic cell growth, tumor angiogenesis, and metastasis, thereby promoting the development of late-life cancers. Indeed, elimination of SNCs with aging attenuates tumor formation in mice, raising the possibility that senolysis might be an effective strategy to treat cancer (*2*).

Given that our knowledge of SNCs in vivo is limited, how should researchers identify senolytic drug targets? One strategy is to identify vulnerabilities shared by cancer cells and SNCs and then use tailored variants of anticancer agents to target such vulnerabilities to selectively eliminate SNCs. Cytotoxic anticancer agents have considerable limitations, including the emergence of therapy-induced resistance due to the high mutation rate of cancer cells and the need for the complete eradication of cancer cells to achieve disease remission. These same challenges are unlikely to occur with senolytic drugs for several reasons. Although evidence is emerging that SNCs are subject to genomic instability, they do not proliferate, thereby precluding the propagation of therapy-resistant clones. Additionally, although rates of senescence increase with aging, the absolute numbers of SNCs that accumulate in tissues remain generally low. Moreover, cancer cells need to be entirely eradicated for successful treatment. In mice, partial (60 to 80%) elimination of SNCs can prevent or attenuate age-associated disease phenotypes (*1*, *2*). Although cancer therapeutics that interfere with cell division are unsuitable as senolytic drugs, agents that block the pathways that cancer cells rely on for survival might be worth pursuing as senolytics. For example, resistance to apoptosis (a form of programmed cell death) is a feature shared by cancer cells and SNCs.

Proof-of-principle evidence for the effectiveness of this strategy comes from targeting the B cell lymphoma 2 (BCL-2) protein family members: BCL-2, BCL-XL, and BCL-W. These antiapoptotic proteins are frequently overexpressed in both cancer cells and SNCs (see the figure). Two targeted cancer therapeutic agents, ABT-*263* and ABT-*737*, have been shown to selectively eliminate SNCs in mice by blocking the interaction of BCL-2, BCL-XL, and BCL-W with BCL-2 homology 3 (BH3) domain–containing proapoptotic proteins (*5*, *6*). BCL-2 inhibitors are senolytic across species, in multiple cell types, and in SNCs resulting from multiple senescence-inducing stressors. In mice, pharmacological inhibition of BCL-2 family members eliminates various kinds of senescent stem cells, including hair follicle, skeletal muscle, and hematopoietic stem cells. This results in rejuvenation of the stem cell populations, presumably by restoring the stem cell microenvironment (niche) (*5*, *6*). In mouse models for two major age-associated human diseases, atherosclerosis and neurodegeneration, ABT-*263* cleared SNCs from atherosclerotic plaques and brain tissue, respectively, substantially attenuating the progression of key disease phenotypes (*7*, *8*).

Another example of learning from oncology pertains to the apoptotic p53 pathway. Molecules that interfere with the interaction between the E3 ubiquitin ligase MDM2 (which negatively regulates p53) and p53 increase p53 activity and thereby induce apoptosis in cancer cells that express wild-type p53. Treatment of a murine osteoarthritis model with the drug UBX0101, which interferes with this regulatory mechanism, triggers apoptosis of SNCs that accumulate in articular cartilage and synovium—cells that are causally implicated in the development of osteoarthritis (*9*).

In addition to the challenge of drug resistance, most cancer therapies are limited by toxic side effects. This is also the case for BCL-2 inhibitors, which reduce the number of neutrophils (neutropenia) and thrombocytes (thrombocytopenia). However, because SNCs accumulate slowly and are nonproliferative, their abundance might be controlled by intermittent dosing, which could prevent side effects from developing. Similarly, drugs that target p53-MDM2 binding are selective but not specific for cancer cells and SNCs. Therefore, some normal cell populations are likely to be affected, which may cause side effects.

Various other approaches have been used to trigger the elimination of SNCs, including the treatment of SNCs with a peptide drug designed to interfere with the interaction of p53 and forkhead box protein O4 (FOXO4), thereby directing p53 to mitochondria for induction of apoptosis (*10*). The creation of galacto-oligosaccharide–coated nanoparticles can selectively deliver cytotoxic agents to SNCs that are positive for the senescence

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marker β-galactosidase (*11*). Moreover, natural products with anticancer properties, such as quercetin and fisetin, and quercetin in combination with the pan-tyrosine kinase inhibitor dasatinib, have been reported to eliminate SNCs in vitro and in mice (*12*, *13*). Although quercetin, fisetin, and dasatinib are often referred to as senolytics, it should be noted that they each act on a myriad of pathways and mechanisms implicated in diverse biological processes. This makes it difficult to decipher how these drugs eliminate or otherwise impact SNCs and to attribute any therapeutic or detrimental effects they may have in clinical trials to senolysis.

Senolytic drugs that inhibit targets originally discovered in oncology could yield promising first-generation drugs to treat humans. However, this strategy may not accomplish the long-term goal of developing ideal senolytics that selectively, safely, and effectively eliminate SNCs upon systemic administration (see the figure). Efforts to identify such "next-generation" senolytics could nonetheless benefit from general principles that have been used in anticancer drug discovery. For instance, it will be important to focus drug development on age-associated degenerative diseases in which SNCs are clear drivers of pathophysiology and in which senolysis could be disease modifying (e.g., osteoarthritis and atherosclerosis). SNCs implicated in conditions that meet these requirements should be characterized to identify any distinct mechanisms that underlie their survival. Agents that selectively target such mechanisms are likely to have the lowest risk of side effects.

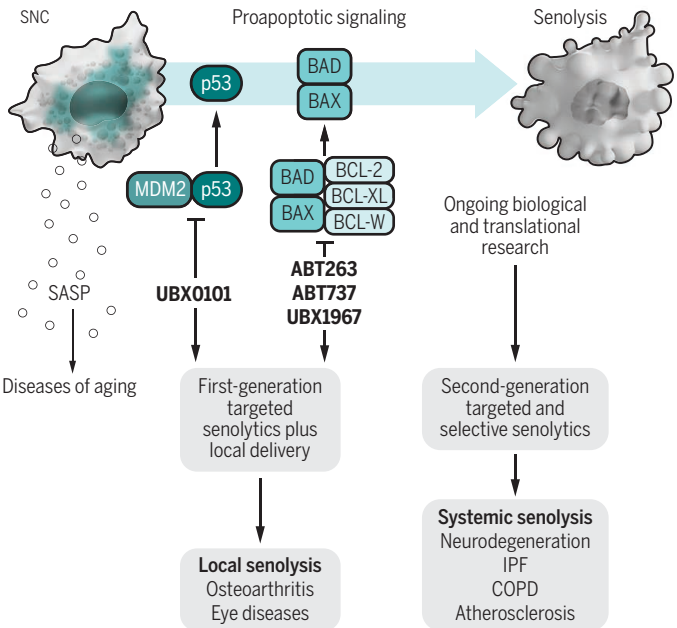
The development of next-generation targeted senolytics will require a richer molecular description of the in vivo properties of SNCs. This includes potentially beneficial SNCs that arise during tissue repair and regeneration to facilitate tissue remodeling and limit fibrosis through the SASP factors that they produce (*14*). One problem in acquiring such information is that SNC-specific markers in vivo are only now being developed. Until such markers are validated, accurate visualization, collection, and tracking of SNCs in tissues and organs and at sites of age-associated disease is difficult. The same challenge also applies for experiments designed to determine whether properties of SNCs in vitro are preserved in vivo and to identify which cells become senescent in a particular disease of aging. Additionally, it is increasingly likely that, rather than being a static endpoint, cellular senescence is a series of progressive and phenotypically diverse cellular states that are acquired after initial growth arrest (*3*). This SNC evolution is at least partially driven by processes that introduce genomic diversity, such as the formation of micronuclei and activation of retrotransposons (*15*). These findings predict that SNCs are heterogeneous collections of cells with fewer shared core properties than anticipated.

The SASP is considered the driver of tissue deterioration and disease, but several fundamental questions regarding the bioactive secretome of SNCs in vivo remain unanswered. For instance, it is unclear to what extent SASP factors systemically drive age-associated pathologies by entering the circulation. If this is substantial, local clearance of SNCs at specific disease sites may not provide therapeutic benefit, and systemic senolysis may be required. Additionally, cell culture experiments indicate that the composition of the SASP varies by cell type and senescence-inducing stressor. The extent to which diversity in SASP composition translates into phenotypic heterogeneity in disease and aging remains to be established. Moreover, the SASP is complex and consists of hundreds of secreted proteins, raising the question of whether the phenotypes result from the effects of many factors or a subset.

As knowledge of the fundamental biology and vulnerabilities of SNCs expands, the rational design of targeted senolytics is expected to yield therapies to eliminate SNCs that drive degeneration and disease. This positive outlook is based on successes in oncology and because the main limitation of cancer therapies—the clonal expansion of drug-resistant cells—does not apply to SNCs. Additional confidence comes from the recent progress in bringing senolytic agents into clinical trials. The first clinical trial is testing UBX0101 for the treatment of osteoarthritis of the knee. Another drug, UBX1967, a BCL-2 family inhibitor specifically tailored for diseases of the aging eye, is also advancing to human testing. Multiple clinical trials treating diverse diseases of aging with senolytic drugs are expected to follow soon. This includes two-step cancer treatment approaches whereby malignant cells are first forced into a senescent state by one drug and then eliminated with a senolytic agent. Success in these first clinical studies is the next critical milestone on the road to the development of treatments that can extend healthy longevity in people. ■

Senolytic therapies for optimized aging

Senescent cells (SNCs) resist apoptosis by activating prosurvival pathways and inhibiting proapoptotic pathways.



First-generation senolytics
Drugs developed to target key components of prosurvival pathways eliminate SNCs and thereby the SASP that drives diseases of aging.

Second-generation senolytics
Development of selective senolytics that safely and effectively eliminate SNCs upon systemic administration awaits further understanding of SNCs.

BAD, BCL-2–associated agonist of cell death; BAX, BCL2-associated X; BCL-2, B cell lymphoma 2; COPD, chronic obstructive pulmonary disease; IPF, idiopathic pulmonary fibrosis; SASP, senescence-associated secretory phenotype.

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ACKNOWLEDGMENTS

J.M.v.D. is a cofounder of Unity Biotechnology and an inventor on patents licensed to or filed by Unity Biotechnology. J.M.v.D. is supported by the Glenn Foundation for Medical Research and NIH grants R01CA096985 and R01AG057493. J.M.v.D. thanks B. Childs, N. David, and D. Baker for helpful discussions.

10.1126/science aaw1299

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Science **364** (6441), 636-637.
DOI: 10.1126/science.aaw1299