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Journal Club

Senotherapeutics: Different approaches of discovery and development

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Aging—a natural process associated with a decline in physiological functions—leads to various age-related diseases, including cardiovascular diseases, cancer, neurodegenerative diseases, and immune system disorders (Guo et al., 2022). Cellular senescence, the hallmark of aging, is defined as a state of irreversible cell cycle arrest associated with stress-induced cellular damage. Senescent cells have antagonistic pleiotropic effects; while beneficial (involved in tumor suppression or wound healing) early in life, they are harmful (causing chronic inflammation or tumor development) in old age (Gems, 2022). During aging, senescent cells accumulate and promote the secretion of senescence-associated secretory phenotype (SASP) factors, including proinflammatory cytokines (mainly interleukin-6 and interleukin-8), chemokines, and matrix-degrading proteases. To ameliorate age-dependent pathological features and expand lifespan, antiaging therapeutics (senotherapeutics) have been developed—(1) senolytics, which selectively eliminate senescent cells and (2) senomorphics, which suppress SASP secretion. Below, we review recent studies on senolytics and senomorphics.

Characteristically, senescent cells have increased resistance to apoptosis stimuli. The discovery of the first-generation senolytics was hypothesis-driven using a mechanism-based approach of selecting agents to target the antiapoptotic pathways. Various proteins (eg, B-cell lymphoma 2 (BCL-2) family proteins, heat shock proteins) or pathways (eg, p53 and phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signaling pathways) regulate apoptosis. The senolytics ABT-737 and ABT-263 inhibit these BCL-2 family proteins to induce apoptosis of senescent cells (Ryu et al., 2023). In addition, natural flavonoids such as quercetin (Q) and fisetin act as senolytics by suppressing the PI3K/Akt cell survival pathway, while dasatinib (D) is senolytic by inhibiting the Src/tyrosine kinase (Nambiar et al., 2023). In a study, Wang et al. (2022) used a popular preclinical senolytic combination therapy, comprising dasatinib and quercetin (D + Q), to eliminate cells highly expressing p21 in visceral adipose tissue (VAT) of obese individuals. They found that while mice transplanted with vehicle-treated human VAT showed glucose intolerance and insulin resistance, those transplanted with D + Q-treated human VAT were insulin sensitive and exhibited alleviation of detrimental metabolic effects.

Lately, second-generation senolytics are being discovered using the alternative approach of targeting markers that are increased in senescent cells. Senescence-associated β-galactosidase (SA-β-gal), for example, is a lysosomal enzyme that accumulates in senescent cells. Shi et al. (2023) designed an SA-β-gal targeting senolytic agent, KSL0608-Se, which was combined with multiple technologies to enhance accuracy and tractability. For instance, constructing a biorthogonal receptor triggered by SA-β-gal helped anchor KSL0608-Se to senescent cells and reduce off-target effects. Additionally, by replacing the oxygen atom with a selenium (Se) atom, rendered KSL0608-Se phototoxic. Thus, KSL0608-Se became photosensitive in the presence of SA-β-gal and killed senescent cells in a dose-dependent manner when irradiated with light. KSL0608-Se treatment with photodynamic therapy reduced the expression of senescence markers such as γ-H2AX, p53, and p21 in the liver and kidney of doxorubicin-induced senescence and naturally aged mice models.

Another strategy for eliminating senescent cells relies on cell surface molecules that are highly expressed in senescent cells. For example, the expression of natural killer group 2 member D ligands (NKG2DLs) is increased in senescent cells, but not in normal cells. Senescent cells expressing NKG2DLs can evade natural killer cell-mediated immune clearance, leading to their accumulation (Pereira et al., 2019). In a related study, Yang et al. (2023) demonstrated that chimeric antigen receptor (CAR) T cells targeting NKG2DLs are potential senolytics in aged mice and nonhuman primates. They infused engineered mouse-NKG2D-CAR T cells into the tail vein of X-ray irradiated mice, resulting in the reduction of NKG2DLs expression in the adipose and muscle tissues, and alleviation of aging-associated pathologies. Similar changes were observed in the liver and lungs of naturally aged mice treated with mouse-NKG2D-CAR T cells. As expected, administering human-NKG2D-CAR T cells eliminated senescent cells in the adipose tissues of rhesus and cynomolgus macaques (nonhuman primates).

Recently, artificial intelligence-driven approaches are being harnessed to discover novel small-molecule senolytics (Wong et al., 2023). Hypothetically, because first- and second-generation senolytics are cell-phenotype specific, machine learning algorithms based on phenotypic information—similar to those used in the discovery of new antibiotics—are applicable to finding novel senolytics. Therefore, Wong et al. (2023), carried out phenotypic screening combined with AI-driven modeling to discover novel senolytics. They first screened senolytic activities of 2,352

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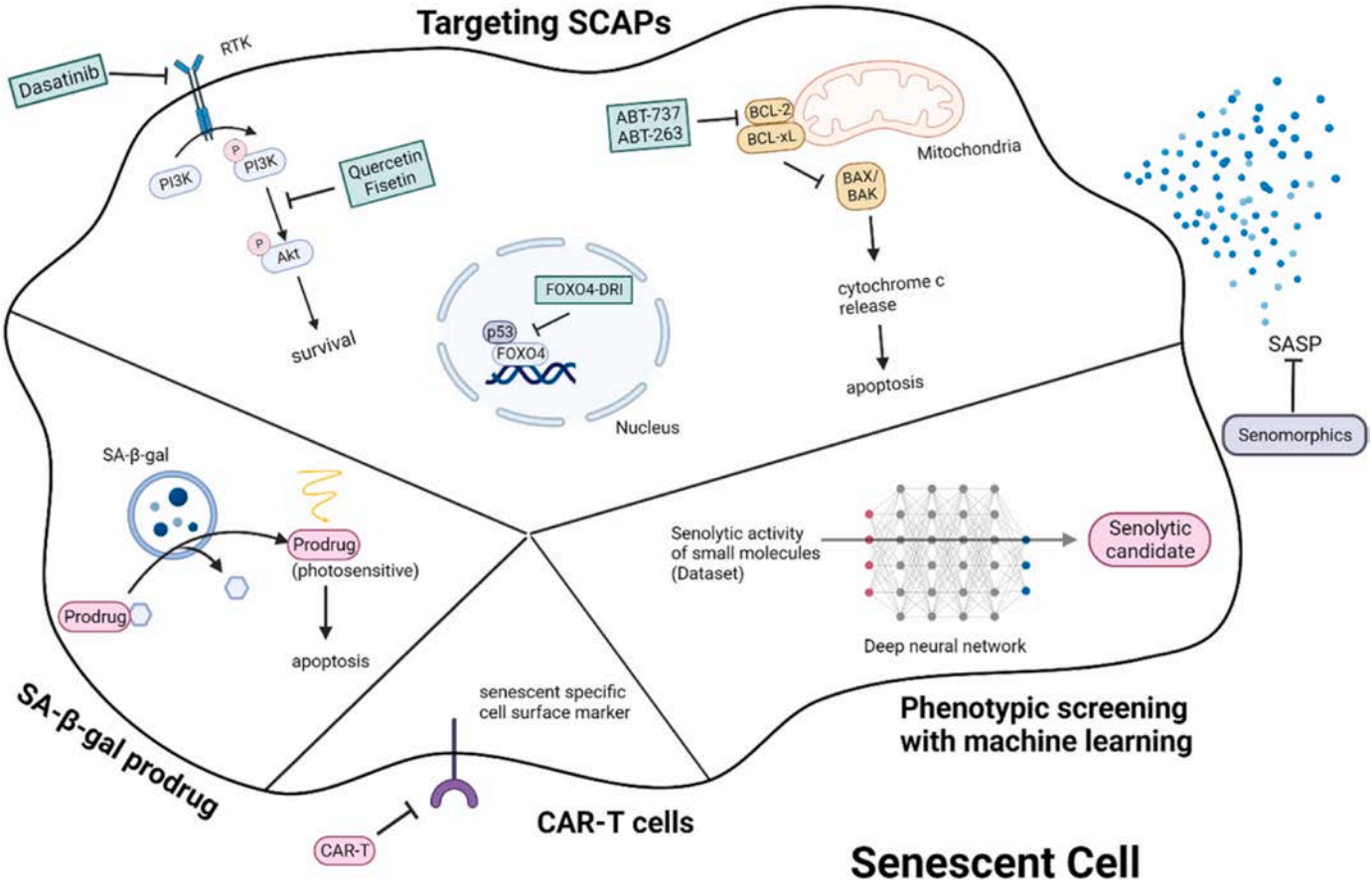
compounds in etoposide-induced senescent cells. They used this dataset to train message-passing graph neural networks to predict senolytic activity of a molecule based on its chemical structure. Subsequently, they screened >800,000 compounds using their trained model and filtered potential senolytics for favorable pharmacokinetics and structural novelty. Three candidate compounds that had high therapeutic indices were found to selectively kill therapy- or replication-induced senescent cells. Moreover, the kidneys of aged C57BL/6J mice injected with one of these compounds had significantly decreased SA-β-gal-positive area and p16 and p21 messenger ribonucleic acid (mRNA) expression compared with those in vehicle-treated mice.

Senomorphics are an alternative therapeutic approach to target cellular senescence. Senomorphics attenuate the senescent phenotypes by inhibiting SASP-inducing signaling pathways, including mammalian target of rapamycin (mTOR), Janus kinase/signal transducer and activator of transcription (JAK/STAT), nuclear factor kappa B (NF-kB), and p38 pathways. For example, metformin, known to suppress SASP production by inhibiting NF-kB, prevented age-associated ovarian fibrosis in mice (Landry et al., 2022). Metformin treatment shifted subpopulations of fibroblasts in the aging ovary; positive remodeling myofibroblasts were increased in the metformin group, whereas SASP-producing fibroblasts were elevated in the aged group. Thus, metformin prevented ovarian fibrosis in aged mice by modulating fibroblast proportions.

As discussed above, senotherapeutics alleviate age-related pathologies in preclinical studies, suggesting these agents may be the key to delaying morbidity and increasing health span. However, the clinical safety profile and efficacy of senotherapeutics must be further verified. All possible side effects must be assessed using longer observation time before clearing them for clinical use.

In summary, we have reviewed the different approaches used to discover and develop senotherapeutics that delay or attenuate age-related diseases. The first senolytic drugs were discovered by targeting the antiapoptotic pathways in senescent cells, whereas novel senolytics were discovered by specifically targeting senescent cell markers. In recent studies, senolytics were developed by integrating multiple technologies. Accordingly, the future discovery of new senotherapeutics may be boosted by using approaches that either—(1) combine several technologies to minimize off-target effects, or (2) screen and evaluate small molecules using deep learning methods.



Senolytics selectively eliminate senescent cells, whereas senomorphics suppress SASPs. First-generation senolytics target the senescent cell antiapoptotic pathways, including receptor tyrosine kinase, PI3K/Akt, p53 and BCL-2 family members. Second-generation senolytics target SA-β-gal or cell surface markers that are expressed highly in senescent cells. Recently, multiple strategies, including library screening and deep learning, are being utilized to discover novel senolytics. RTK: receptor tyrosine kinase, SCAP: senescent cell antiapoptotic pathway, SASP: senescence-associated secretory phenotype, CAR-T: chimeric antigen receptor T cell.

AUTHOR CONTRIBUTIONS

J.H.Y. conceived the study and wrote the manuscript. H.J.H. conceived the study, supervised the project, and secured funding.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

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